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A COMPARISON OF THE INCLUSION BODIES OF FOWL-POX AND MOLLUSCUM CONTAGIOSUM *

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With the demonstration of the infectious nature of the inclusion bodies of fowl-pox [1] it has seemed possible that inclusion bodies of other filtrable virus diseases might be used in similar experiments. Among these diseases is molluscum contagiosum, which closely resembles fowl-pox with regard to the large size and the distinctness of its intracytoplasmic inclusions. Furthermore, in a recent study of the subject, Goodpasture and King [2] have confirmed the observations of Lipschütz [3] and da Rocha Lima [4] that the inclusions of molluscum contain myriads of extremely minute round bodies — another respect in which molluscum and fowl-pox inclusions are similar. This observation, together with the fact that the molluscum bodies are of such large size, encouraged us in the hope that these bodies might be freed by tryptic digestion for purposes of inoculation, as were the fowl-pox inclusions. Accordingly, a series of experiments was undertaken, the results of which will be reported briefly. Since the gross and microscopic characteristics of molluscum contagiosum have been adequately described in textbooks and numerous papers on the subject, the molluscum body, which is the most characteristic feature of the lesion, may be considered without further description of the infected tissue.

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The Effect of Trypsin on the Molluscum Bodies

When a piece of tissue from a molluscum lesion is placed in 1 per cent trypsin, the shell of epithelium about each molluscum body digests away, freeing the body (Fig. 1). As may be seen, the bodies thus freed by digestion are oval or round in outline and exhibit a finely granular structure. In addition to this fine granulation, a sort of trabeculation of the bodies can be distinguished especially clearly in a preparation which has been teased out in saline (Fig. 2). These trabeculae were described by White and Robey [5] in 1902, the entire structure being aptly compared to a honeycomb. The authors described the formation of the trabeculae from the cytoplasm of the affected cell. That this explanation is true and that the trabeculae are actually a portion of the epithelial cell is indicated by their reaction to trypsin. A period of thirty minutes in the digesting solution causes the trabeculae to dissolve and allows the various compartments to disintegrate (Figs. 3 and 4). The molluscum bodies still retain their outline and consist of a jelly-like matrix in which the appearance of granulation is conspicuous. The matrix material, after digestion of the trabeculae, is extremely sticky and strings out like a piece of dough when it is touched with a glass or steel point. Active Brownian motion of granules may be seen in the gelatinous compartments, and the granules are easily expressed and many of them become free while still vibrating rapidly.

In their reaction to trypsin the molluscum bodies differ markedly from the firm, compact inclusion bodies obtained by the digestion of a fowl-pox lesion. The fowl-pox inclusions are protected from the action of trypsin, presumably by their lipoid content, and can be digested completely free from adventitious cellular material without interfering with their homogeneity. In order to get molluscum bodies similarly free from cellular material, the removal of their cytoplasmic trabeculae is necessary. With this removal the molluscum bodies are found to be composed of sticky masses and are rendered quite unfit for any sort of manipulation as units. Because of this stickiness, our attempted duplication of the inoculation experiments which were successfully performed in the case of fowl-pox had to be abandoned. Recently a technique has been evolved for inoculating minute portions of the fowl-pox inclusions.[6] This latter technique has not yet been attempted with molluscum, but we feel

that it would be practicable, and preferable to attempts to inoculate the entire inclusion bodies.

The gelatinous matrix of the molluscum bodies shows a much greater resistance to the digestive action of trypsin than is shown by the trabeculae. Furthermore, in any fresh preparation, whether the bodies are suspended in trypsin, normal saline or distilled water, the matrix exhibits the markedly granular appearance which has already been mentioned. This appearance is caused by the presence of innumerable Lipschütz granules — minute coccoid structures 0.25 of a micron in diameter within each inclusion. The granules were discovered by Lipschütz in smear preparations stained with Loeffler's flagella stain. He also observed them within the molluscum bodies in fresh preparations. At the present time the Lipschütz granules are recognized by many observers to be as characteristic of the molluscum lesion as are the molluscum bodies themselves, for, wherever the latter are found, there the Lipschütz granules can also be demonstrated. The granules are resistant to the action of trypsin to an even greater degree than the matrix material which surrounds them. In our experience, periods of five hours digestion have made no demonstrable change in their morphology and in all probability they would resist tryptic digestion for much longer periods.

The Effect of Distilled Water

One of the striking characteristics of the inclusion bodies of fowl-pox is the marked swelling which they exhibit when placed in distilled water. It has been pointed out that this swelling continues until the original volume of the inclusions is increased two or three times.[6] Molluscum bodies, on the other hand, show little or no swelling under the influence of distilled water. The matrix of the molluscum body is probably completely permeable, since it promotes no swelling, while the lipoid material of the fowl-pox inclusion acts as a semipermeable membrane, permitting a marked imbibition of water.

Within the fowl-pox inclusions which have become swollen in distilled water may be seen myriads of minute bodies dancing about in rapid Brownian motion. These bodies, called Borrel bodies after their discoverer, are an exact counterpart of the Lipschütz granules of molluscum. In either disease the minute structures are coccoid in shape, 0.25 of a micron in diameter and react in similar fashion to

special stains [7] (Figs. 5 and 6). As may be seen they appear either singly, in diploid or chain form, or in masses. Lipschütz stated that the minute granules are readily differentiated from any impurity or precipitate which might settle on the slide, and, with regard to a carefully made preparation, we can completely corroborate this statement. Moreover, the great uniformity in the size and shape of the granules in the two diseases militates against the argument of those who contend that such granules are mere fragments of chromatin or keratohyaline material.

The most effective method of obtaining the minute elements well dispersed is, in the case of fowl-pox, to allow the digested inclusions to become swollen in a pool of distilled water on a slide and then allow the water to dry.[6] The relatively great tension of the thin film of water abruptly tears apart the lipoid material of the inclusion at the moment the water dries from its surface, leaving the Borrel bodies nicely dispersed (Fig. 6).

The inclusion bodies of molluscum not only show no imbibition when they are placed in distilled water, but, possibly because of their failure to swell in water, fail completely to be broken up when they are allowed to dry out of distilled water. The best preparations of the Lipschütz granules are obtained by making direct smears from the molluscum lesion, or by grinding the lesion, as will be described later.

Effect of Trituration

A study of the literature indicates that there is a difference in the readiness with which the fowl-pox and molluscum viruses may be passed through filters. Marx and Sticker,[8] and later Juliusberg [9] were successful in filtering fowl-pox virus through Berkefeld filters. Burnet [10] was able only occasionally to obtain passage through a Berkefeld filter and in no case through a Chamberland filter. Juliusberg,[11] on the other hand, obtained molluscum virus after passage through a Chamberland filter, and Wile and Kingery,[12] apparently without difficulty, passed active molluscum virus through a Berkefeld filter. It would seem that the molluscum virus filters somewhat more readily than does the fowl-pox virus.

Since the common mode of preparing virus material for filtration is that of thorough grinding in a mortar, we were interested to observe the different microscopic pictures presented by the smears of

ground material in the cases of molluscum and fowl-pox respectively. After grinding bits of molluscum tissue in saline in a mortar for periods of five minutes, smears of the resulting material show the Lipschütz granules to be well dispersed (Fig. 5). After a similar treatment of fowl-pox material the smears show irregular masses of débris with very few discrete, definite Borrel bodies. In the case of fowl-pox, grinding the diseased tissue evidently fails to liberate the Borrel bodies completely, while in molluscum the liberation and dispersion of the Lipschütz granules is practically complete. This difference in the reaction of the inclusion bodies of the two diseases to grinding may again be due to the lipoidal element in the inclusions of fowl-pox.

Comment

Whether or not the readiness with which the Lipschütz granules are dispersed upon grinding the molluscum tissue is of importance in explaining the apparently greater filtrability of this virus cannot be stated definitely at this time. In the case of fowl-pox, evidence has been presented which would associate the Borrel bodies with the actual virus of the disease.[6] While no such evidence is at present available in the case of molluscum contagiosum, still the similarity of the Lipschütz granules to the Borrel bodies in size, shape and position within the inclusion points to the probability that the minute elements in the two diseases represent the viruses concerned. If such is the case, the ready dispersion of the Lipschütz granules upon grinding and the great difficulty met with in attempting to disperse the Borrel bodies afford an explanation of the relatively greater filtrability of molluscum.

Though the great similarity of Borrel bodies to Lipschütz granules has been pointed out, there is no intention in so doing of indicating a common etiology of the two diseases. The reverse has been demonstrated by numerous observers, and, in our own hands, the inoculation of fowl-pox on man and of molluscum on chickens has in no case given any suggestion of a positive result. Moreover, repeated intravenous injections of molluscum virus into chickens has failed, in our experience, to induce any immunity to subsequent infection with fowl-pox virus. That fowl-pox, particularly, is a highly specific disease is indicated by the fact that a single passage of the closely related pigeon-pox through fowls causes the virus, in most instances,

to lose its virulence for pigeons while still producing the typical fowl-pox lesion in chickens. If strains of the virus are specific for such closely related forms as fowls and pigeons, one would not expect the infectious agent of fowl-pox to cause disease in man. Several attempts to infect monkeys (*M. rhesus*) and other laboratory animals with molluscum virus have in our hands proved unsuccessful.

Summary

1. The inclusion bodies of molluscum contagiosum may be freed from surrounding cellular material by tryptic digestion.

2. Unlike fowl-pox inclusions, the molluscum bodies are found to be sticky and gelatinous after they have been digested. Because of this characteristic they cannot be manipulated readily with the Chambers microdissection apparatus.

3. The gelatinous matrix of the molluscum bodies has a markedly granular appearance due to the presence within it of myriads of Lipschütz granules — minute coccoid structures 0.25 micron in diameter. These granules are identical in size, shape and staining reactions with the Borrel bodies of fowl-pox. They are resistant to the action of trypsin.

4. The inclusion bodies of molluscum, on being placed in distilled water, show little or no swelling. Under similar conditions the fowl-pox inclusions swell markedly, due, probably, to their lipoid material acting as a semipermeable membrane.

5. Trituration of the molluscum inclusions readily breaks them up into the component Lipschütz granules. Fowl-pox inclusions, similarly treated, fail to break up so readily into Borrel bodies. This difference in the reaction of molluscum and fowl-pox to trituration may afford an explanation for the relatively greater filtrability of the former.

6. Fowl-pox and molluscum contagiosum are apparently specific for fowls and man respectively, cross-inoculation experiments having proved unsuccessful. Attempts to transfer molluscum to monkeys and other laboratory animals were also unsuccessful.

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DESCRIPTION OF PLATE

PLATE 1

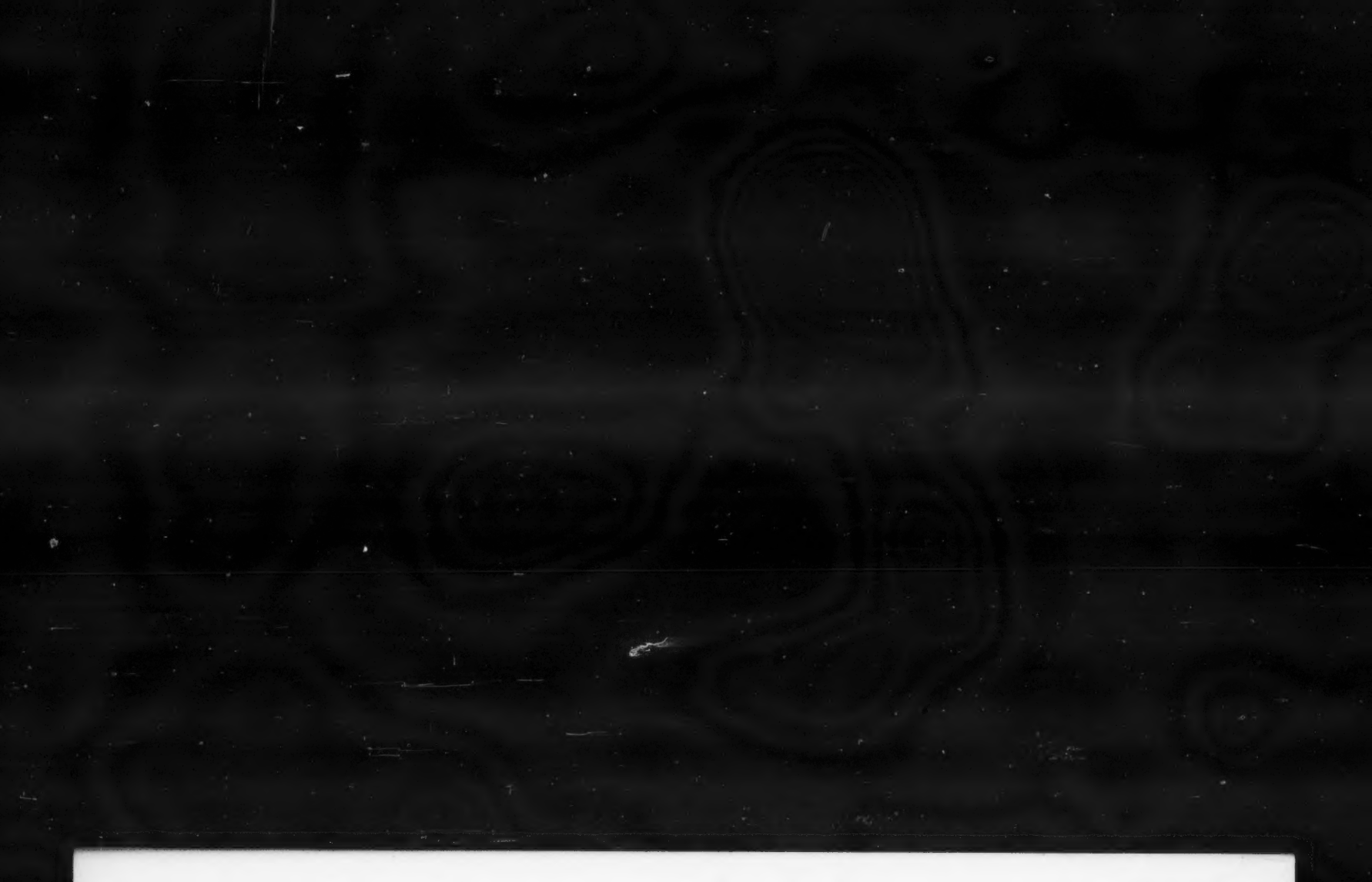
FIG. 1. Inclusion bodies of molluscum contagiosum (molluscum bodies) freed from cells by tryptic digestion. × 550.

FIG. 2. Molluscum body in normal saline, teased free from surrounding cells. Note trabeculation within the inclusion. × 1450.

FIGS. 3 and 4. Molluscum bodies in trypsin with beginning digestion of trabeculae. Note finely granular appearance of matrix material. × 1450.

FIG. 5. Lipschütz granules of molluscum contagiosum. Morosow's stain. Photomicrograph taken with white light. × 1860.

FIG. 6. Borrel bodies of fowl-pox. Morosow's stain. Photomicrograph taken with white light. × 1860.



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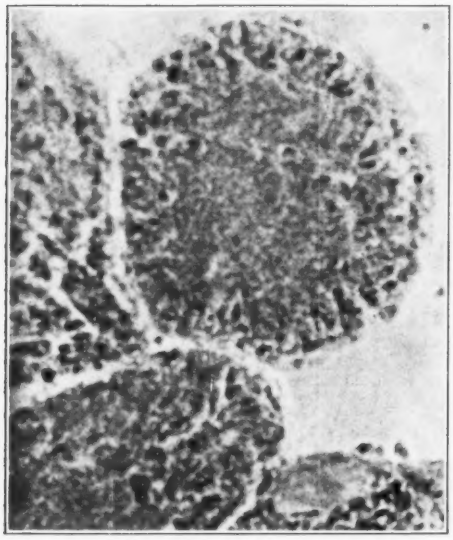

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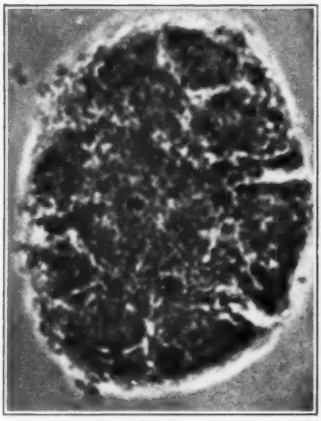

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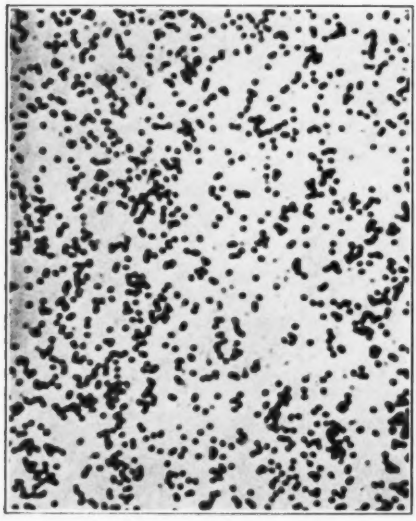

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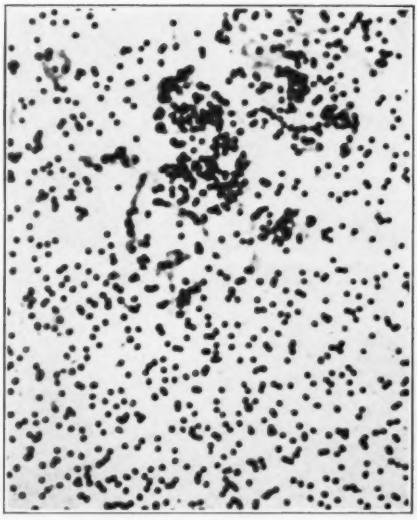
Goodpasture and Woodruff



6

Fowl-Pox and Molluscum Contagiosum

TRANSFORMATION OF SINUSOIDAL ENDOTHELIUM INTO THE ORDINARY CAPILLARY TYPE *

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According to a prevalent view the capillaries of certain organs are lined by cells unrelated to ordinary endothelium. This conception is based largely on the common observation that the endothelium of the spleen, liver and bone marrow is actively phagocytic under natural and experimental conditions, in contradistinction to endothelium elsewhere which usually remains inactive. A related important characteristic of the endothelium of these organs is its affinity for colloidal dyes experimentally administered. In subcutaneous granulation tissue the author [1,2] found that the endothelium may be artificially stimulated to such a degree that it behaves in both of these particulars like the sinusoidal type. That all endothelial cells are potentially sinusoidal was concluded. The present experiments were aimed at a lessening of the functional activity of sinusoidal endothelium and a determination of any variation in phagocytic capacity. Other investigators (Bloom [3] and Higgins and Murphy [4]) have marked the Kupffer cells with particulate matter and subsequently observed the development of inflammatory lesions.

Method

Granulation tissue was first produced and at intervals its endothelial cells tested for phagocytic capacity. Foci of granulation tissue in the liver of rats were produced in two ways. In one set of experiments a thick suspension of sterile casein was injected through a large needle with trochar lumen into the liver, and in a second series the liver margin was crushed with forceps. The vascularized lesions had much the same appearance except for the presence of numerous foreign body giant cells in the animals injected with casein. Also, accumulations of neutrophilic leucocytes appeared about the larger

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casein deposits. In both sets of rats individuals were killed on the third, fifth, and seventh days. One to five hours preceding death, 0.5 cc. India ink was injected into the tail vein.

Description of Experimental Lesions

In the sections from the animals killed on the third day after injection there are necrotic foci with disappearance of hepatic cell nuclei. The sinusoidal endothelial cells are here and there preserved, and the blood spaces which they line are continuous with the capillaries in the adjoining granulation tissue. Likewise, in some of the sections from animals killed on the fifth day, the necrotic cells are incompletely dissolved. Immediately about the necrotic lobules it is not always possible to determine whether the capillaries are persisting sinusoids or are capillaries newly formed in the granulation tissue. The position and direction of the capillaries in some of the locations show them to be new angioblastic tissue, and although mitoses are not numerous, several mitoses in interstitial cells may be seen in occasional single oil immersion fields. As regards carbon content, contrast of necrosis and granulation tissue foci with distant uninvolved portions of the liver is striking (Fig. 1). In the experimental lesions where the endothelium is no longer in contact with living hepatic cells there is relatively little phagocytosis (Fig. 2). In the rats killed on the seventh day, and in some at the end of five days, the hepatic cells are completely autolyzed. In these sections some collagenous material has appeared in the granulation tissue. In the intact lobules carbon is abundant, but phagocytosis is slight or absent where the sinusoids pass into the granulation tissue. This is true although the channels are obviously continuous.

Discussion

The question as to whether there are two distinct types of blood-vascular endothelium often is not clearly expressed in the discussions dealing with it. Maximow [5] has always stated his views definitely on this point. He believes that the sinusoidal (littoral, histiocytic) type is not true endothelium and has a separate embryonic origin: "The study of the embryonic histiogenesis of the connective tissue and blood shows, indeed, that the resting wandering cells (his-

tiocytes) of the embryo originate largely through the transformation of the lymphocytoid or histioid wandering cells into quiescent forms" (p. 177). He ascribes a dual origin, monocytic and histiocytic, to the migrating mononuclear phagocyte or polyblast. Most observers agree that the Kupffer cell is a normal integral part of the vascular lining of the liver. Eliot,[6] however, maintains that the histiocytes are merely monocytes temporarily anchored in the lumina of the capillaries of certain organs. That monocytes are present here in varying numbers is not disputed, but both the morphological and experimental evidence show that phagocytic cells are an essential part of the capillary walls. These permanently fixed phagocytes concern us in this discussion.

Do the Kupffer and other phagocytic cells that line the hepatic sinusoids differ from ordinary endothelium because of an unrelated embryonic origin according to Maximow's view, or is the difference the result of environment? The part played by the endothelium in metabolism is, in many particulars, ill understood. As emphasized by Aschoff,[7] it participates in lipoid metabolism. In cholesteremia it undergoes profound structural change. In the larger blood vessels the function is at a minimum with slight passage outward of blood constituents. No doubt the function varies with the organ. In the lungs, with relatively little parenchyma, the transfer is chiefly gaseous and under normal conditions this endothelium shows slight phagocytic properties. In the liver, adrenal and pituitary, the endothelium is a bridge between blood and parenchyma and the liver especially is extremely active as regards phagocytosis. In the spleen and bone marrow, the cells that line the capillaries perform a normal phagocytic function especially concerned with the disposal of worn-out red corpuscles. Endothelium in these organs not only provides a smooth lining for the vascular network but also functions in the removal from the blood of particulate matter and colloids. In human pathology, and especially in destructive liver lesions, instances are not infrequently seen in which the hepatic endothelium in a new environment loses its phagocytic property. By the experimental destruction of the hepatic cells the capillaries that are newly formed, or those that represent persisting sinusoids, assume after a time the appearance of capillaries in granulation tissue elsewhere and lose much of their capacity for ingesting particulate matter. In the normal liver as the capillaries leave the periportal tissue to penetrate the

lobule the endothelium establishes contact with the hepatic cells and assumes an added function. When this function is lost, the cells revert to the usual inactive type.

Summary and Conclusions

1. In granulation tissue within the liver, the capillaries may arise in the usual way by angioblastic proliferation, or sinusoids may persist as capillaries. In both instances the vessels are continuous with the sinusoids in the adjoining lobules.

2. With loss of contact with living hepatic epithelium the endothelium becomes less phagocytic.

3. The peculiarities of sinusoidal endothelium depend on its functional activity, which, in turn, is determined by its normal histological position. From the behavior of sinusoidal endothelium present in granulation tissue, it appears not to be a distinctive type of cell.

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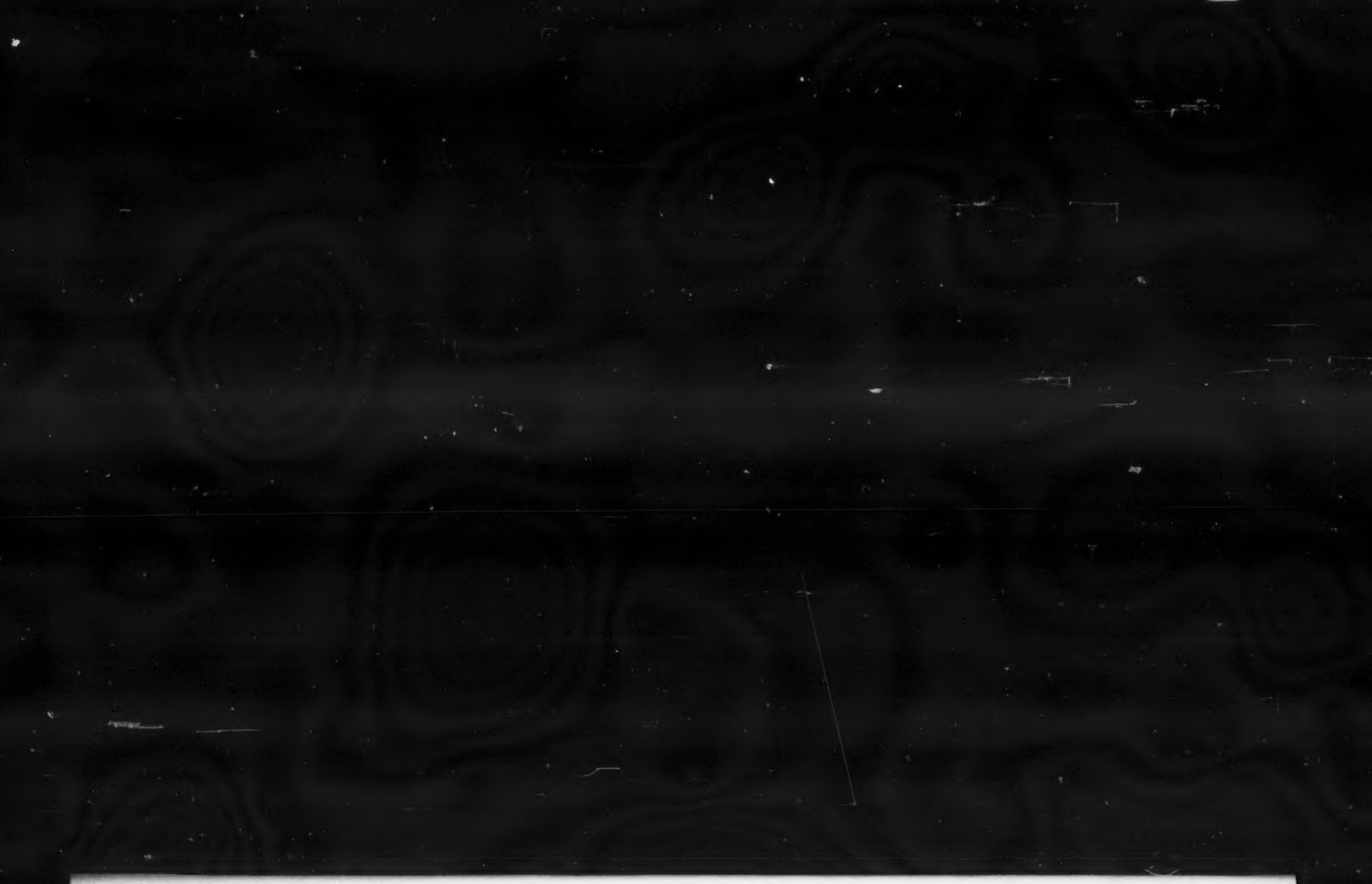
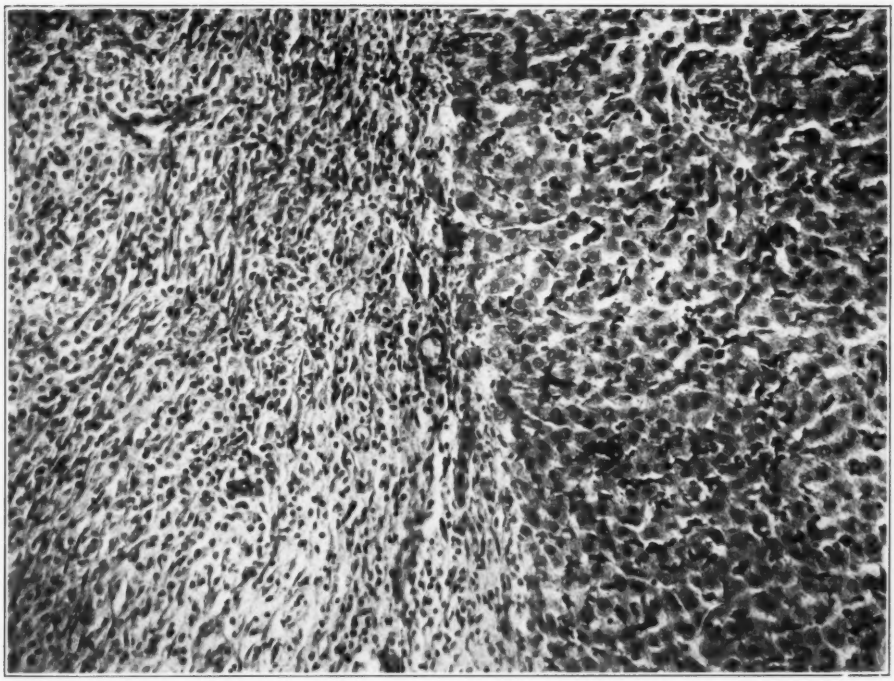
DESCRIPTION OF PLATE

Plate 2

Fig. 1. Sinusoidal endothelium within liver lobules packed with ink particles. The capillary endothelium of the highly vascularized granulation tissue is practically devoid of carbon. Five-day granulation tissue in liver of white rat. × 200.

Fig. 2. Endothelial cells of the sinusoids with huge amounts of ingested carbon. Endothelium of abundant capillaries of the granulation tissue shows little phagocytosis. In granulation tissue in the liver the phagocytic activity on the part of the vascular endothelium is no greater than that of granulation tissue in general that has not been subjected to artificial stimulation. Granulation tissue five days old. × 500.





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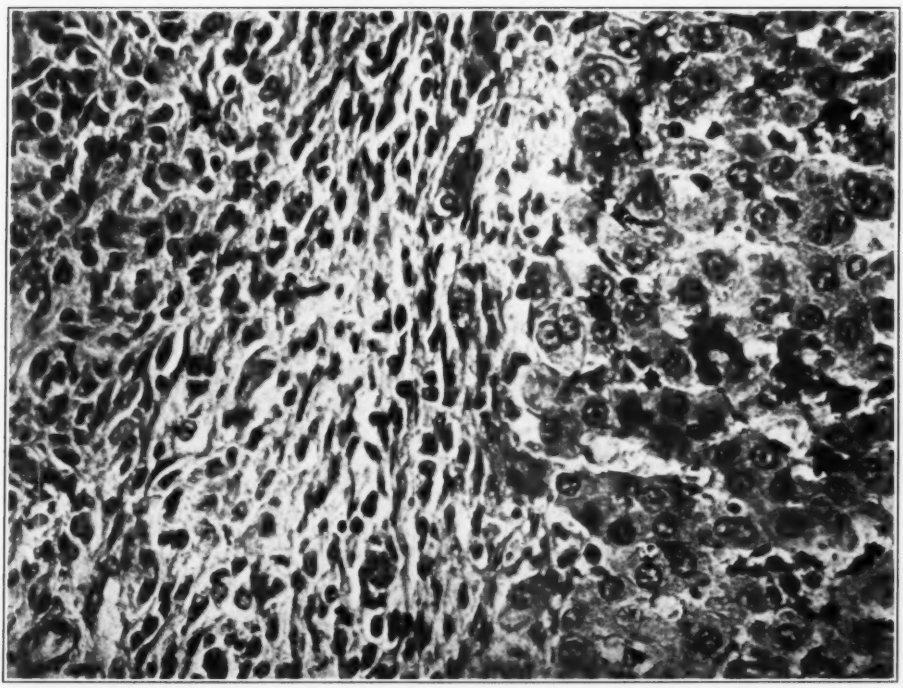

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McJunkin Transformation of Sinusoidal Endothelium

BENIGN POLYMORPHOCELLULAR TUMORS OF THE KNEE REGION *

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The somewhat variable, but specific structure and benign clinical course of a group of slowly growing tumors arising from tendon sheaths and aponeuroses, commonly in the hands, much less frequently in the feet, and very rarely in other locations such as the knee, have been recognized since the first descriptions of the growths by Gross,[1] Paquet,[2] Reverdin,[3] and Heurtaux.[4] These early French writers were much impressed by the presence in the tumors of large giant cells ("myeloplaxes"), resembling those of epulis and other benign bone neoplasms. Heurtaux distinguished the newly described tumors from sarcoma by the clinical results in the cases and termed them "myelomas." This name although obviously a misnomer, has persisted almost to the present day. Such tumors are comparatively rare, as evidenced by the fact that Spiess [4] was able to collect only forty-five instances from the literature up to 1913. The peculiar complex and puzzling structure which characterizes the newgrowths has ever been a matter of considerable interest to clinicians and pathologists in Europe, particularly in France and Germany, so that a fairly voluminous literature on the subject has grown up in the languages of these countries, while comparatively little is to be found in English.

Almost from the beginning there has been controversy as to the nature and pathogenesis of the tumors. Some writers, beginning with Fleissig [5] in 1913, have contended that these peculiar tumors are inflammatory granulation growths although others have always regarded them as true, but benign neoplasms. Lecène and Moulonguet [6] compare the tumors to changes resulting from chronic irritation of long duration, such as occur in old hematocele, hernial sacs and chronic bursitis. Talbot, who cites these two authors, speaking of tumors with myeloplaxes occurring in the articular

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synovial membranes, raises the question as to whether these are tumors at all, and is inclined to regard them as granulomas. Very recently Frangenheim [7] also has expressed the opinion that xanthomatous giant cell tumors in the knee joint are inflammatory granulation growths.

The not infrequent history of trauma preceding the appearance of tendon sheath and joint capsule or synovial tumors has led many to suspect injury and inflammation as important predisposing agents. Broders [5] obtained a history of trauma in 35.3 per cent of his seventeen cases of xanthomatous tumors of tendon sheaths which he believes are probably the result of extravasation of blood following injury or infection, and present more of the features of a granulation tumor than a true neoplasm. Wegelin [8] concludes that xanthomatous giant cell tumors of joint capsules owe their origin on the one hand to a local mechanical factor (trauma), and on the other to a hypercholesteremia.

A local or general imbalance of lipoid metabolism has been assigned an etiological rôle by others, notably McWhorter and Weeks [9] who consider all forms of xanthoma, including those arising from tendon sheaths, a result of systemic disease with hypercholesteremia as the essential feature. They feel that these nodules are not true neoplasms but the result of an irritative connective tissue reaction to the deposition of cholesterin. Although blood cholesterol values are lacking in the majority of recorded cases of tendon sheath and synovial tumors, normal readings have been obtained in a few instances (Frangenheim,[7] Gonzalez-Aguilar [12]).

The recent investigations of Wile, Eckstein and Curtis [10] cast serious doubt upon the validity of previous views concerning the importance of hypercholesteremia in the formation of skin xanthoma at least. They found only 1.5 to 2 per cent of cholesterol in xanthomas of the skin, much less than the amount present in normal epithelium and circulating blood, and feel that some cause other than the previously accepted high blood cholesterol must operate for the development of the tumors. Apparently responsible is a disordered fat metabolism in which cholesterol as a constituent of the body lipids has a rôle. Wile concludes that because of the frequency of xanthoma without lipemia and of lipemia without xanthoma, the knowledge of the xanthomatous state is still incomplete.

The multiplicity of names which from time to time have been ap-

plied to the type of tendon sheath, aponeurotic and synovial tumors under consideration — myeloma, myeloid tumor, xanthosarcoma, pigment-containing giant cell sarcoma, fibrohemosiderin sarcoma, gigantocellulare xanthomatodes and xanthomatous giant cell sarcoma — to cite only a few, emphasizes their multiform and complex structure which, according to Ewing [11] presents four main features: (1) masses of cells predominantly or exclusively spindle or polyhedral-shaped, enclosed by a dense connective tissue, at times quite cellular and lacking in giant cells but of benign character; (2) groups of so-called xanthoma cells containing an abundance of doubly refractive lipoid substance and at times so numerous as to be the counterpart of xanthoma of the skin; (3) usually numerous and very large multinucleated giant cells, and (4) hemosiderin pigment-containing tumor cells.

The tumors show a marked predilection for the flexor tendon sheaths and palmar aponeurosis of the hand. Forty-one of the forty-five cases collected by Spiess [4] occurred in this member, and of these thirty were on the palmar surface.

Ewing states that the growths are commonly well demarcated, firm, opaque, yellowish or yellowish brown, markedly lobulated, readily removed by operation and although in some instances rapid enlargement takes place, the usual history is that of a slow growth requiring many years to attain the size of an egg. Recurrence after complete extirpation is rare.

The tendons about the knee joint seem to be singularly free from sheath tumors, but one has recently been reported by Gonzalez-Aguilar.[12] The growth was the size of a mandarin orange and enveloped the patellar ligament completely. The joint cavity was not opened. The mass was found to be well encapsulated, mostly yellowish with a few almost white spots, and contained small islets of a dark brown color. The usual variety of cells found in tendon sheath tumors were observed in microscopic preparations.

According to Smith [13] primary tumors in the region of joints may originate from several sources, namely: (1) in relation to the synovial membrane of the joint cavity itself; (2) from the synovial membrane of overlying bursae; (3) fascial aponeuroses, and (4) from tendons and tendon sheaths. Buxton [14] however, states that primary tumors of tendon substance proper are extremely rare and probably do not occur.

Although rarely the site of primary tumor formation, the capsules and synovial membranes of joints give rise to a variety of neoplasms including hemangioma, lipoma simplex or arborescens, chondroma, osteoma, malignant sarcomas, and finally the so-called xanthomatous giant cell tumors. Hartman [15] was able to collect only sixteen examples of capsular and synovial tumors from the literature prior to 1922, to which he added another. The knee was the seat of involvement in fifteen of the seventeen cases. Garrett [16] reports four instances and Harbitz [17] eleven from his own material, ten of which occurred in the knee while the other involved the wrist. Although some one hundred and twenty cases of tendon sheath [illegible] and synovial membrane tumors had been record[illegible] recorded in the literature prior to 1922, Hartman [15] [illegible] discovered that in spite of exhaustive study no one had previously compared or identified the tumors arising from the synovial membranes of tendon sheaths and those primary in the synovia of joints. He distinguishes three groups of synovial tumors: (1) pedunculated tumors; (2) growths with giant cells, and (3) diffuse tumors without giant cells. He would class the growths with the benign tumors of connective tissue origin, although there is a potential malignancy which may be enhanced by palliative measures and incomplete excision. He feels that these neoplasms should not be termed sarcoma unless clinical or pathological evidence of malignancy appears. By reason of the fact that any one of the characteristic cells listed by Ewing may be absent from an otherwise typical case, Hartman prefers the name of myeloid tumor above others.

A search through the available references since Hartman's review in 1922 has shown the need for bringing the literature concerned with xanthomatous giant cell tumors of the knee joint up to date, and this will be attempted as briefly as possible. Instances of frankly malignant sarcoma, as well as benign neoplasms other than the type mentioned above will be omitted.

Summary of Cases

Garrett's study,[16] which appeared two years after Hartman's publication, seems to have been overlooked by recent European writers.

Two of Garrett's cases are without clinical records and only the pathological diagnosis is given. One showed foam cells, capillary

angi[illegible]oma, giant cells and pigment, the other was called a giant cell fi[illegible]oma, hemorrhemangioma or xanthoma. The third example was that reported by Hartman. Case 4 was a woman, aged 21 years, who had a painful and swollen knee for six months prior to operation. Necrotic tissue was found below the patella and the affected substance was removed. Microscopic examination disclosed numerous giant cells at first suggesting giant cell tumor of bone, but the cells were embedded in a different type of tissue, were smaller and spindle-shaped. Endothelial-lined blood spaces were present. There were no foam cells. Although the growth was not removed completely the patient was well six years later.

In Cabot's Case Records [18] in 1926 appears an account of a tumor of known etiology, in some respects simulating the type under consideration. A male 24 years of age, suffering from acute infectious arthritis of the knee was at first treated by injection of 2 per cent formalin in glycerin. Arthrotomy with removal of a tumor three years later disclosed a growth closely associated with the entire synovial membrane, of rough fibrous consistency with roughening and erosion of bone and cartilage. Microscopically the structure consisted of small mononuclear wandering cells with newly formed blood vessels, a fibrous stroma forming an intercellular substance in which single cells were embedded. A previous biopsy section had disclosed numerous foreign body giant cells. The histological appearance bore some resemblance to a giant cell tumor. The growth was considered a chronic inflammatory process.

Harbitz [17] in 1927 listed the following nine cases of tumors originating in the articular capsule and synovial membrane of the knee or adjacent areas.

"*Case 1.* In a woman, aged 51, a tumor, the size of a pigeon's egg, developed in the capsule of the knee in the course of two years. It was firm, gray-white, fibrous, not distinctly limited and microscopically a polymorphocell sarcoma, with giant cells in some places, and containing some pigment.

Case 2. In a woman, aged 66, a tumor, the size of a walnut, was removed from the capsule of the knee joint. It was firm and hard, with a gray-white, cut surface; microscopically, it proved to be a small cell sarcoma. In the following four years it did not recur.

Case 3. A woman, aged 27, for some years had had a swelling in the recessus superior genu, which microscopically proved to be a flat, firm, fibrous, gray-white tumor, a sarcoma fusocellulare.

Case 4. Ostensibly after an injury in a man, aged 26, a peduncular tumor the size of an almond developed in the synovial membrane of the knee joint, a sarcoma fuso-giganto-cellulare, microscopically.

Case 5. Ostensibly after an injury to the knee, small tumors developed on the outer side of the capsule in a woman, aged 39. Microscopically this appeared to be a polymorphocell sarcoma.

Case 6. A patient had a recurrent polymorphocell sarcoma on the inner side of the knee joint capsule. Death resulted from return of the growth.

Case 7. In a woman, aged 71, a large flat sarcoma, fibro-giganto-cellulare, formed in the capsule of the knee joint in the course of five months.

Case 8. In a man, aged 22, a sensitive neurofibroma, the size of a bean, developed in the capsule of the knee joint in the course of two years.

Case 9. A sailor, aged 58, presented a calcareous fibroma, the size of a goose egg, which had existed for thirty years on the inner side of the knee."

Harbitz believes these growths form a special and separate group, less well defined than those originating from tendon sheaths, but connected with them by transitions. He regards the structure of articular capsule and synovial newgrowths as more purely sarcomatous than those derived from the sheaths of tendons, containing fewer giant cells, little pigment and no xanthomatous tissue. In my opinion Cases 8 and 9 do not properly belong in the group of capsular and synovial tumors. In addition to the neoplasms already mentioned, Harbitz describes another, the clinical history of which was furnished by Dr. Sundt.* The growth occurred in a young woman 20 years of age, whose right knee became swollen after a blow. A year later a cast was applied for supposed tuberculosis, without benefit. On opening the joint a little bloody fluid, a thickening and reddish brown discoloration of the capsule were noted. There was some thinning and honeycombing of the articular surface of the femur. The microscopic pathological diagnosis was probable xanthofibrosarcoma gigantocellulare pigmentosum. Signs of inflammation were lacking and nothing suggested the organization of extravasated blood or exudation. The tumor much resembled the xanthosarcomas of tendon sheaths and like them appeared comparatively benign, although giving some evidence of destruction of cartilage and bone.

Abadie [19] in 1928 reported two rare tumors of the knee, one primary in the synovial membrane, the other a patellar bone tumor diagnosed "tumeur à myeloplaxes like certain types of epulis." Only the first will be mentioned. A woman 45 years of age, had for several months noted a small mass on the external surface of the right knee. Operation revealed a small discoid growth attached to the synovial

* Sundt, H. (*Norsk. mag. f. lægevidensk.*, 1929, **90**, 521) has redescribed this case in detail, together with an instance of chondromatosis of the knee. He states that the woman was well four years after the operation.

membrane, necessitating excision of a small portion of it. The growth was of leather-like consistency, café au lait colored, with some dark yellow patches. Histological examination showed a type of granuloma with a predominance of typical lymphocytes, but also including multinuclear giant cells and ochre-colored pigment both free and within macrophages.

The tumor described by Talbot [6] occurred in the left ankle of a man, 20 years of age. Following a fracture and refracture four years before, treated by traction at the ankle, a swelling appeared. Operation disclosed an irregular tumor 5 by 3 by 2 cm. lying partly in the outer plane of the capsule but arising mostly from the inner surface of the synovial membrane. The color varied from saffron yellow to ochre. The conclusion reached after microscopic study was: "a connective tissue tumor, a granuloma with giant cells which microscopically would have been called sarcoma a few years ago." Iron-containing pigment was present also. Improvement was noted.

Talbot describes in some detail the case of Paitre and Bruas (*Soc. de méd. mil. franç., Bull. mens.*, Nov., 1923).

A cavalryman had injured his left knee in a fall one year before with only temporary impairment of function. A new fall brought him to Paitre and Bruas who found a localized swelling the size of an apricot pit which seemed to enucleate itself from the depths of the articulation and appeared to be situated on the patellar ligament and inseparable from it. A tumor the size of a mandarin orange was enucleated. The mass was implanted with the fat pad in the bottom of the intercondylar notch. A good functional result followed the operation. The pathological diagnosis was: "fibrosarcoma with myeloplaxes, tumor histologically benign."

Wegelin [8] has seen one instance of benign xanthomatous giant cell tumor in a 43 year-old man, who for eight years had noticed a cyst on his knee clinically having the appearance of a joint-mouse. At operation a pedunculated mass was found and excised. The pedicle of the growth was attached to the joint capsule beneath the patella; the mass was lobulated, firm, grayish red, in places yellowish, and measured 3.5 by 2 by 0.5 cm. Sections exhibited a richly cellular growth, for the most part spindle-shaped and with abundant cytoplasm. Irregularly distributed throughout were numerous multinucleated giant cells. A fine collagenous intercellular substance separated the tumor cells. Extra- and intracellular brownish pig-

ment, large and small groups of fatty cells containing little doubly refractive substance, and clefts covered by endothelial-like, cubical or low cylindrical cells completed the picture. There was no recurrence after ten years.

Wegelin's second case was a complex structure of distinctly sarcomatous nature which he believes may have been the result of a differentiation of cells from a newgrowth of the xanthomatous type.

Other cases mentioned by Wegelin follow:

Weil (*Berl. klin. Wchnschr.*, 1915, **52,** pt. 1, nr. 6, 129), and Seyler (*Virchows Arch. f. path. Anat.*, 1922, **239,** 20) each mention xanthomatous tumors of the joint capsules, but their descriptions as to the exact location of the growths are not clear.

Züllig (*Cor.-Bl. f. schweiz. Aerzte*, 1917, **47,** 1368). A woman 34 years of age, complained of pain and marked effusion in the right knee without previous trauma. Several pedunculated tumors which extended into the joint cavity were removed. Microscopically these were found to consist of spindle, polymorphous and giant cells with rich deposits of cholesterin in many places imparting a xanthomatous appearance. Recurrence had not taken place one year later.

Wustmann (*Deutsche Ztschr. f. Chir.*, 1925, **192,** 381). For several years following an injury of the left knee a woman 42 years of age had what appeared clinically to be a joint-mouse. At the operation a polypoid, polycystic, chestnut-sized tumor, microscopically exhibiting many blood vessels, xanthoma cells and giant cells was found. Wustmann concluded that the growth was derived from the vascular endothelium.

Herxheimer (*Arch. per le sc. med.*, 1927, **50,** 201, Festschr. f. Morpurgo) mentions a yellowish proliferative villous synovial tumor of the knee joint in a woman of 30 years. Spindle cells, giant cells and xanthoma cells were observed in microscopic preparations.

Frangenheim's paper [7] is based upon observations in a man of 50 years. Nineteen years before, the left knee had been dislocated, followed by effusion and later subsidence of symptoms. After nineteen years a swelling appeared, accompanied by very severe pain. A firm brownish mass the size of a hen's egg, in part broadly attached to the synovial membrane, and partly pedunculated, was removed surgically. Histological examination by Professor Dietrich showed a tumor composed of round and polygonal cells separated by a fine fibrillar stroma, numerous giant cells, blood and pigment deposits,

and having much the appearance of a giant cell sarcoma of the epulis type.

Five years later, and six months before coming to Frangenheim's clinic, the growth reappeared, accompanied by pain and effusion from the medial surface of the knee and popliteal space. On incising at the site of the previous operation, bloody fluid escaped from the joint cavity. The entire synovial membrane was covered with brownish villi of varying lengths. It was necessary to remove the anterior part of the joint capsule. A hygroma lying in the popliteal space and lined by the same villous structures as the growth in the knee was excised at the same time.

Professor Siegmund, who studied the sections, reported a new-growth of granulation tissue type, richly vascular, distinguished by the presence of giant cells, the formation of a homogeneous osteoid intercellular substance, groups of iron-containing cells and lipoid. He regarded the growth as a benign resorption tumor belonging in the class of so-called giant cell sarcoma of tendon sheaths. Six months later the patient had no symptoms referable to the knee. Frangenheim states that the presence of osteoid intercellular substance has never before been observed in this type of tumor and that such a finding makes the similarity of structure to that of epulis still greater.

Frangenheim cites the following authors not mentioned by Wegelin.

Tobler (*Beitr. z. klin. Chir.*, 1927, **140**, 545). The tumor arose from the medial meniscus, was cherry-sized, flat, yellowish red, in places brownish and had a thin pedicle 3 mm. long originating from the joint capsule.

Albertini (Gutartige Riesenzellgeschwulste, G. Thieme, Leipzig, 1928) without clinical data, mentions a free joint body of the knee which he called a xanthosarcoma.

Simons (Neue deutsche Chirurgie, 1928, **43**, F. Enke, Stuttgart). A man of 50 years stated that six months before his right knee had been injured in a fall against a sharp edge. For the last six weeks the pain had been severe and lightning-like, accompanied by swelling of the joint. A firm body could be felt, disappearing on flexion and reappearing on extension. A disc-shaped mass, the origin of which was difficult to determine and at first thought to be a meniscus, was discovered at the operation. Histologically it consisted of xanthoma

cells and a sarcomatous-like basal structure. Pathological diagnosis: xanthosarcoma.

Négrié and Canton (*Bull. et mém. Soc. nat. de chir.*, 1929, **55**, 617). A male, 20 years of age, was admitted to the hospital with a diagnosis of articular foreign body in the right knee. Three years previously he had suffered a severe injury of this knee, necessitating bed rest for three months. One year later the knee would swell suddenly on the slightest traumatism. A voluminous, hard and easily movable object giving the impression of a foreign body could be felt along the medial margin of the knee. Flexion caused it to disappear. Arthrotomy disclosed a growth at the level of the prespinal surface of the tibia and adherent to the synovial membrane. The object removed looked like a pebble, slightly excavated on one side, protuberant on the other, and measured 4 by 3 by 2 cm. Microscopic examination revealed a myeloplaxoma having a framework of connective tissue showing a discrete inflammatory reaction, traces of blood pigment in the process of resorption and large multinuclear giant cells. The patient was discharged as cured after six months.

These two authors saw two cases but the other is not described. They regarded the growth as benign and the result of an inflammatory reaction rather than a tumor in the true sense of the word.

Case Reports

The material here presented was obtained from a father and his daughter both of whom were operated upon by Dr. Harry C. Blair. A clinical study is being prepared by Dr. Blair and will be published separately.

Case 1. Mrs. H. E. P. (29272), age 31 years. The patient was entirely well until a year ago when her right knee was injured by striking it against a bench. Sudden and severe pain followed by swelling forced her to remain in bed for a week. Thereafter the knee appeared normal for a time but six months ago a gradually increasing swelling appeared above the patella. The knee never catches, movement is not limited, pain is slight and absent at night. No other joints are involved.

Inspection revealed no apparent enlargement of the affected knee but on palpation a small mass which seemed to roll beneath the vastus medialis muscle was made out. The movements of flexion and extension were normal. There seemed to be a small amount of fluid within the joint cavity. Roentgenogram was negative. A blood cholesterol estimation was not done.

The patient consented to operation. An incision along the medial aspect of the thigh over the region of the quadriceps bursa exposed a tumor mass which was found to make up a portion of this bursa but lay mostly within the main articular cavity of the knee. The intra-articular portion had a peculiar "medusa-head" appearance. The growth was removed without difficulty, the edges of the capsule were coapted, the postoperative course was uneventful and the patient was walking without aid after a month. Eight months later she was still well.

Gross Examination of Specimen: The tissue consists of a very ragged soft appearing but firm mass measuring 12 by 5 by 2 cm. in its maximum diameters, and weighing 35 gm. Along one border is a thin layer of adipose tissue, and adjacent to this is a shiny, yellowish white membranous area, possibly representing a portion of synovial membrane, but in the main the growth is made up of a fairly firm faintly yellowish to yellowish white substance devoid of visible blood vessels. Extending irregularly through the tumor are many wavy, light yellowish brown to brownish streaks producing a somewhat laminated effect. Extending outward from one border are numerous thread- and band-like extensions, some only a fraction of a millimeter in diameter, others as much as 5 mm. in thickness. For the most part these form bridges between the main tumor and another similar but smaller mass, but some have one free end. The longest band is formed by the union of two smaller ones and on its opposite end there is suspended an irregularly outlined brownish structure identical in appearance with the main growth.

Microscopic Examination: Sections from different parts of the specimen reveal a peculiar histological structure. In the main, the growth seems to be made up of numerous closely packed cells of variable size and shape having a faintly reticular, foamy-appearing and abundant cytoplasm. Some are round or oval, others polygonal or spindle-shaped. Their nuclei are on the whole rather small, pyknotic and generally centrally situated. The growth is much more vascular than the gross appearance would indicate. Scattered throughout are numerous small and collapsed, or large and distended capillary blood vessels, together with occasional arterioles. Three distinct changes occur about the blood vessels; in some instances these consist of radially arranged collars of small oval-shaped cells having hyperchromatic nuclei; others are surrounded by irregular zones of phagocytic cells laden with both finely and coarsely granular yellowish brown pigment giving a strong Prussian blue reaction;

still others are collared by lymphocytes. These areas correspond to the softened brownish zones observed macroscopically. Occasional multinuclear giant cells are found in the vicinity of the pigmented areas and blood vessels, but are nowhere numerous and in the fatty cell areas are absent altogether. The giant cells vary considerably as to size and the number of nuclei within them; their nuclei are closely packed, lie toward the central part of the cells and in many respects resemble closely those found in benign giant cell tumors of bone. Hemosiderin pigment granules occur in some of these cells. None of the various types of cells show mitosis of their nuclei and nothing suggestive of malignancy is observed. No bursal structure is recognized microscopically.

Sections stained for fat with Sudan III reveal an abundance of small and medium sized bright orange-colored droplets within the cytoplasm of the foamy appearing cells throughout the growth. The pigment-containing cells and giant cells appear dark brownish in these preparations.

Pathological Diagnosis: Benign polymorphocellular tumor arising from synovial membrane of right quadriceps bursa and extending into the main articular cavity of the knee.

CASE 2. Mr. J. S. (30377), age 68 years, father of Mrs. H. E. P., presented himself, walking with the aid of a cane and scarcely limping. He stated that he had worked every day of his life and had always enjoyed good health. Forty-five years ago, while a cadet in an officers' training school in Russia, a horse had kicked his left knee. The resulting disability caused his retirement from the Army and he emigrated to America. After the injury the knee remained swollen and enlarged slowly. During the past three years there has been a more rapid increase in size, especially during the last three months.

The left knee was seen to be greatly enlarged, measuring 90 cm. in circumference, as compared to 39 cm. on the unaffected side. Many large veins were visible under the skin about the knee. Extension was complete and flexion possible to 45 degrees. The foot on the affected side was swollen and edematous. The dorsalis pedis and posterior tibial arteries were palpable.

Amputation was deemed the operation of choice. The patient refused but consented to an exploration and biopsy. A medial incision revealed a large encapsulated mass lying beneath the thinned-out vastus medialis muscle. Bleeding was profuse but sufficient tissue was obtained for pathological examination. An uneventful postoperative course followed, complicated only by the development of a symmetrical edema extending from the umbilicus caudally. Consent for amputation was now obtained and the leg was severed proximal to the tumor mass. The edema disappeared entirely within twenty-four hours and did not recur. The wound healed without infection and after four months the patient was able to walk with the aid of an artificial limb. At the time of writing, seven months after the amputation, there has been no local recurrence or signs of metastases.

Gross Examination: The left leg has been amputated at the junction of the middle and distal thirds of the femur. The knee is enormously increased in size, rounded and lacks the normal contour and anatomical landmarks. The circumference is 70 cm. at the knee, 48 cm. over the calf and 29 cm. at the ankle. There is a marked pitting of the skin on pressure over the entire foot and leg. The skin over the lower third of the leg is dark brownish and scaly but elsewhere is whitish. On the medial surface of the knee is a long, recently healed surgical wound with a reddish scar.

On sagittal section through the femur, knee joint and tibia the subcutaneous fat, muscles and fascia appear to be very edematous. The veins are filled with soft blackish non-adherent clots. The popliteal artery is displaced far posterior to its normal position by a large tumor mass, but its lumen is patent. Entirely surrounding the knee joint and extending for a distance of 25 cm. superiorly, and 20 cm. inferior to it is an enormous, irregularly lobulated growth. The largest single tumor has a maximum diameter of 11 cm. and is situated posteriorly in and above the popliteal space. This mass involves the posterior capsule of the knee joint, extends upward along the periosteum of the femur, then posteriorly into the popliteal space. It is quite irregular in shape but the peripheral border is sharply outlined. The color varies from light yellowish to a rusty brown with the two colors often intermingled. The tissue has a honeycombed appearance in places while other areas are solid and firm. A sticky yellowish substance exudes from the cut surfaces. Situated below the knee and posterior to the tibia are several similar but smaller nodules, one of which has invaded the capsule of the knee. On the anterior surface of the thigh and directly invading and destroying the capsule of the knee joint is another large mass somewhat similar to the one first described, except for the color, which is almost uniformly a deep rusty brown. The knee joint is filled with a soft brownish substance which covers and obscures the medial meniscus. The lateral meniscus is unchanged. In places the cartilage over the femoral condyles is thinned but not entirely eroded. The same is true for the head of the tibia. A somewhat curved area of erosion is observed in the posterior cortex of the patella. The bed of this defect is filled with a brownish substance. Similar-appearing material fills the recessus superior. The growth stops short just below the head of the tibia in the sagittal cut but is found further out in the

soft tissues for some little distance below this point. At the point of attachment of the anterior cruciate ligament to the femur, there is a soft dark brownish area with a solid yellowish border extending for a distance of 1.5 cm. through the synovial membrane and cartilage into the marrow of the femur, where it ends abruptly.

Microscopic Examination: In sections stained with hematoxylin and eosin the character and appearance of the growth are found to vary with the area from which the blocks were selected. Those from the faintly yellowish white zones show rounded or oval cells of rather uniform size and containing small and somewhat pyknotic nuclei. The cytoplasm has a distinct foamy appearance and stains only faintly with eosin. In portions of such areas the cells have undergone partial or complete necrosis. In the brownish zones some of the cells are of the same type as those just described, but the discoloration is undoubtedly due to the presence of a large amount of coarsely granular yellowish brown pigment particles occurring intracellularly for the most part. The pigment is often so abundant as to fill the entire cell cytoplasm, but in some instances the foamy appearing cells contain only a few granules. Other parts of the tumor exhibit a still different picture in that the cells are stellate, polygonal or spindle-like and the cytoplasm is homogeneous and stains well with eosin. The nuclei too are larger, rounded or oval and tend to be hyperchromatic, often with one or two small nucleoli, but exhibit no mitoses. In places the cells are compact but more generally are somewhat separated by clear spaces. Careful search reveals a few large multinucleated giant cells of the foreign body type. Lying here and there in the growth are strands of fascia, fat cells, and small blood vessels which show no tumor cells within their lumina. At the periphery the newgrowth is sharply demarcated from the atrophic muscle and connective tissue cells surrounding it. In sections stained with ferrocyanide-carmine the brownish pigment appears bluish, proving the presence of iron. Sudan III preparations disclose an abundance of varying sized globoid masses in the foam-like cells. In some instances the color is bright orange, in others the substance has a dirty orange-brown hue. Stains for mucin give a negative reaction and Van Gieson's preparations disclose relatively little supportive tissue within the newgrowth. A block of bone taken from a point near the insertion of the anterior cruciate ligament where macroscopic evidence of invasion by the newgrowth is apparent, reveals

many pigment-filled tumor cells lying in the marrow, but even here the line of demarcation is quite sharp and the cells are not more anaplastic than elsewhere in the growth.

Pathological Diagnoses: Large benign polymorphocellular tumor of left knee invading the joint capsule, femur and patella; recently healed surgical wound; healed varicose ulcer of leg with pigmentation of skin; marked edema of foot and leg.

Discussion

The location, macroscopic appearance and histological structure of both tumors herein described conform in every respect to that of the poorly understood, variously termed, but nevertheless definitely recognized group of newgrowths arising usually from the sheaths of tendons and aponeuroses, but also rarely from joint capsules and synovial membranes. Histologically the two growths are identical. Each is richly vascular, contains xanthoma-like cells filled with both unsaturated fat staining well with Sudan III, or a muddy yellowish brown-colored material which is probably cholesterol, multinucleated giant cells largely of the epulis variety, abundant extra- and intracellular pigment giving a positive reaction for iron, and other cells of the most varied morphology.

In Case 1 the growth was comparatively small and its origin was established definitely as coming from that portion of the synovial membrane of the knee joint forming the quadriceps bursa. From here many irregular villous projections extended into the articular cavity proper. The tumor therefore belongs in the pedunculated group of Hartman's classification. The relatively slow growth, comparatively small size of the mass, lack of bone involvement or demonstrable metastatic deposits either prior to, or since the operation, together with the innocent microscopic appearance indicate an essentially benign character.

The newgrowth in Case 2 is most remarkable by reason of the extremely long clinical course of forty-five years and because of its enormous bulk. The latter feature makes this tumor quite unique among others of its kind since it is apparently by far the largest ever reported. Few of the tumors recorded in the literature have been larger than a hen's egg even though present for many years. On account of the great size and extensive ramifications of the growth, it was quite impossible to determine the original point of departure.

However, since the bulk of the mass lay in the soft tissues about the knee with comparatively little tumor tissue within the joint cavity, it is reasonable to assume an extra-articular origin, possibly from one of the tendon sheaths about the knee. In spite of the bulkiness of the tumor, the history of recent rapid enlargement and the obvious evidence of penetration of the joint capsule and destruction of bone, there is no microscopic evidence of a malignant departure. The lack of demonstrable metastases before or after amputation of the leg is further evidence of its benign nature. In the presence of a growth of such generous dimensions one is not surprised to find some destruction of soft tissues and even bone, either as a result of pressure atrophy, repeated hemorrhages, interference with the blood supply or from a combination of one or all these factors. If it were possible to state with certainty that the tumor in Case 2 had an extra-articular origin the present study would offer still further evidence of the now generally recognized fact first pointed out by Hartman that identical newgrowths may arise from the sheaths of tendons and synovia of joints. Unfortunately, for reasons mentioned previously, such proof is lacking in the second case.

The occurrence of identical newgrowths in the same location in closely related individuals is interesting but may be only a coincidence.

Etiologically, trauma appears to have been the immediate exciting cause for tumor formation in both individuals. Although a history of injury is lacking in many of the previously recorded instances of these tumors, it appears to have been definitely associated with the onset of the growths in so many others that one cannot escape the conviction that trauma may play an important etiological rôle.

In the more recent European literature on xanthomatous giant cell tumors of joints there is a distinct tendency to regard the growths as granulomatous lesions rather than true neoplasms. On this point the writer has little to offer except that it is difficult to believe that a granuloma could attain the size of the growth in Case 2. The fact that apparent transition from such growths to frankly malignant sarcoma occurs, as pointed out by Harbitz and Wegelin, is likewise evidence of their being neoplastic rather than granulomatous.

The problem of nomenclature on the basis of cell morphology is a troublesome one because of the variety and variability of component cells in individual cases. Although most of the growths contain at

least some of the cell types mentioned by Ewing, others do not. The tendency has always been to name the tumors according to the most striking characteristic or characteristics, with the result that none of the terms is wholly inclusive or completely satisfactory. The recent tendency on the part of nearly all writers to drop the term sarcoma and to condemn its use is a distinct advance. Hartman's view that such tumors should be classed as benign growths until clinical or pathological evidence of malignancy is manifested is logical and sound. The writer has therefore refrained from calling the two tumors reported in this paper sarcomas. The designation "polymorphocellular tumor" introduced by Harbitz is less misleading and more descriptive than any of the many names by which the tumors have been known in the past, and I have therefore adopted it in preference to others.

Summary

1. Two instances of benign polymorphocellular tumors occurring in the knee region of closely related persons are described.

2. In the daughter, the newgrowth originated in the synovial membrane, a rare site of tumor formation, and was benign.

3. In the father, the tumor was of exceptionally long duration, attained very bulky dimensions and although exhibiting locally destructive properties also appears to have been benign. Because of the large size and extensive ramifications of the mass, the point of origin could not be determined, but certain facts point to an extra-articular source.

4. Most commonly newgrowths of the type herein reported arise from the sheaths of tendons and only rarely from the capsule or synovial membrane of joints. In the latter event the knee is much more frequently involved than any other joint in the body. The rarity of such joint growths is evidenced by the fact that the author was able to collect from the literature, in addition to his own cases, only forty-four instances of closely similar or identical tumors.

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DESCRIPTION OF PLATES

PLATE 3

FIG. 1. Case 1. Photograph of tumor removed from quadriceps bursa and extending into articular cavity of knee. Right: ragged external surface with dependent mass connected with main tumor by a thread-like band. Left: cross-section of growth showing pale xanthomatous areas separated by brownish pigmented zones.

FIG. 2. Case 1. Medium high power photomicrograph illustrating the polymorphocellular character of the tumor. The fine blackish intracellular bodies are hemosiderin pigment. A multinucleated giant cell and capillary blood vessel are included in the field.

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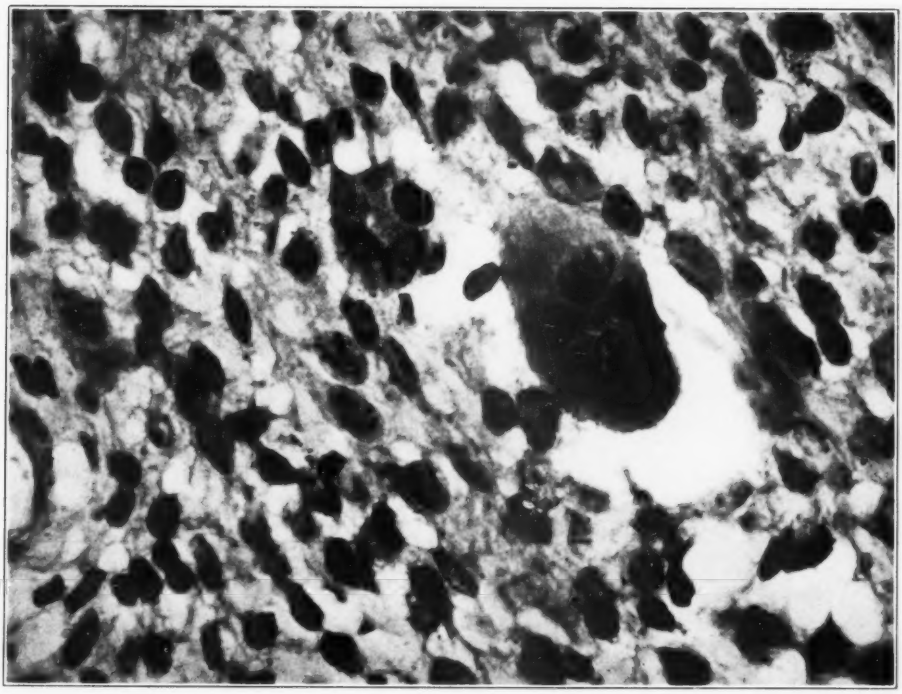


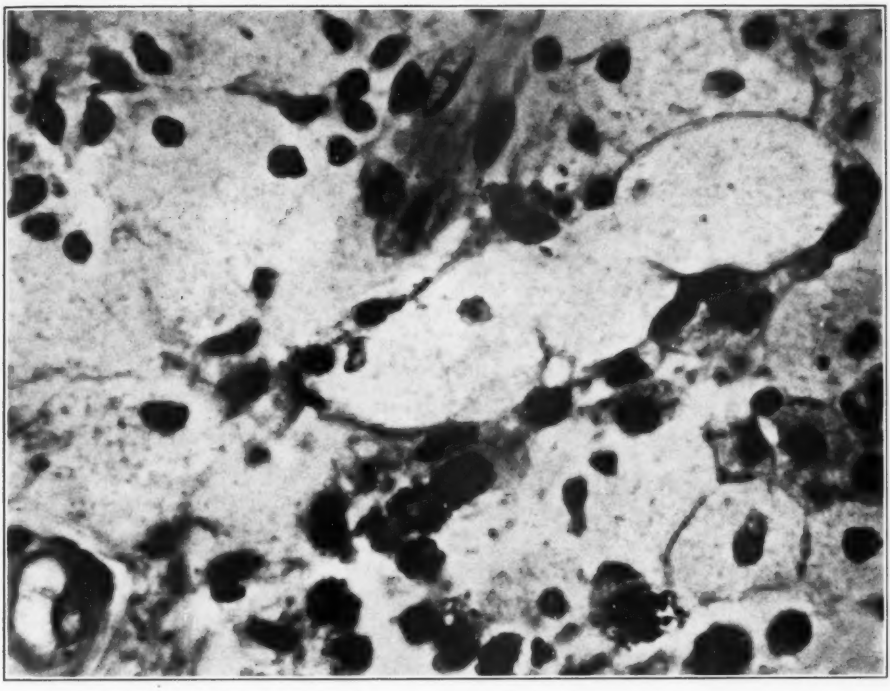
2

Hunter Benign Polymorphocellular Tumors of Knee Region

PLATE 4

FIG. 3. Case 1. High power view from area adjacent to that shown in Fig. 2. Scattered throughout are enormous foamy-appearing cells distended with lipoid. The irregular blackish masses are aggregations of hemosiderin. Several spindle cells occur in the upper center. A single capillary may be seen in the lower left corner.

FIG. 4. Case 2. Photograph. Sagittal cut through left knee showing the macroscopic appearance and location of the newgrowth, the character of which is well shown in the large mass situated in and proximal to the popliteal space. The center is rusty brown and honeycombed, the periphery nearest the femur yellowish white, solid and xanthomatous. The very dark tumors contain a great deal of hemosiderin throughout. Destruction of the joint capsule is evident both posteriorly and anteriorly. In sawing the specimen a part of the medial condyle of the femur was torn away giving the appearance of far more bone destruction than actually existed. The medial meniscus is covered with brownish tumor substance which also fills the recessus superior. An irregular area of erosion is visible in the posterior cortex of the patella.



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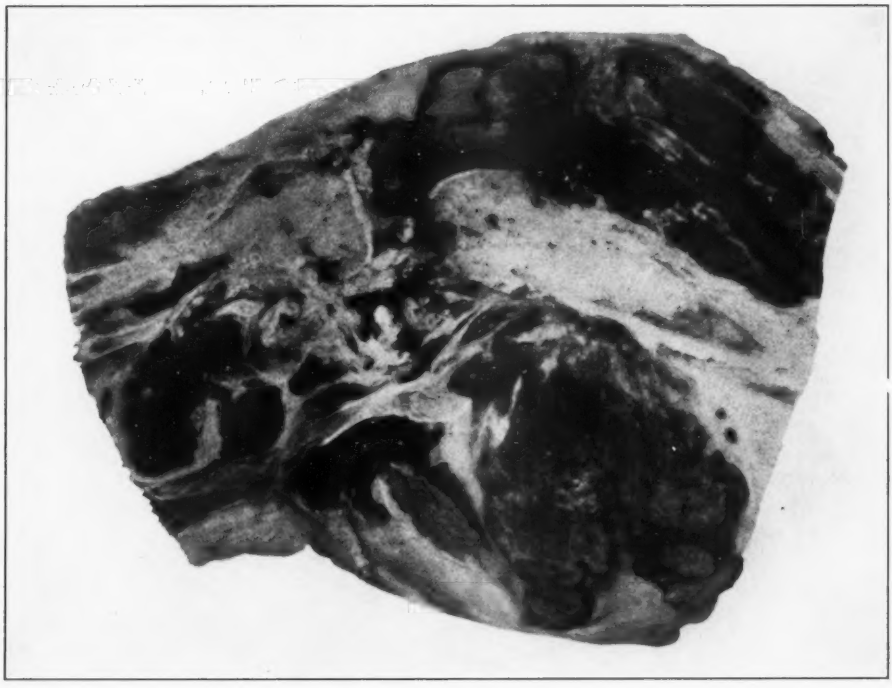

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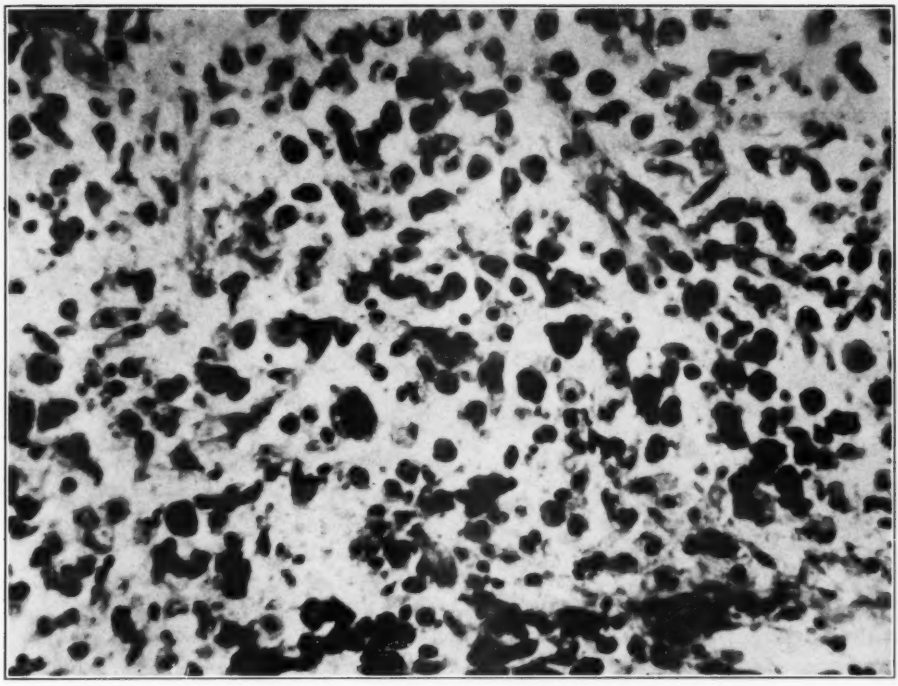
Hunter Benign Polymorphocellular Tumors of Knee Region

PLATE 5

FIG. 5. Case 2. Moderately low power photomicrograph giving a general idea of the varied cell morphology of the tumor. The lightly stained round and oval cells are engorged with fat and cholesterol, the large blackish masses are accumulations of yellowish brown pigment. Scattered spindle cells may be seen here and there. The field does not include any of the giant cells mentioned in the microscopic study.

FIG. 6. Case 2. High power photomicrograph from a portion of the field illustrated in Fig. 5. The majority of the cells have a distinctly vacuolated cytoplasm, all others are heavily impregnated with hemosiderin.



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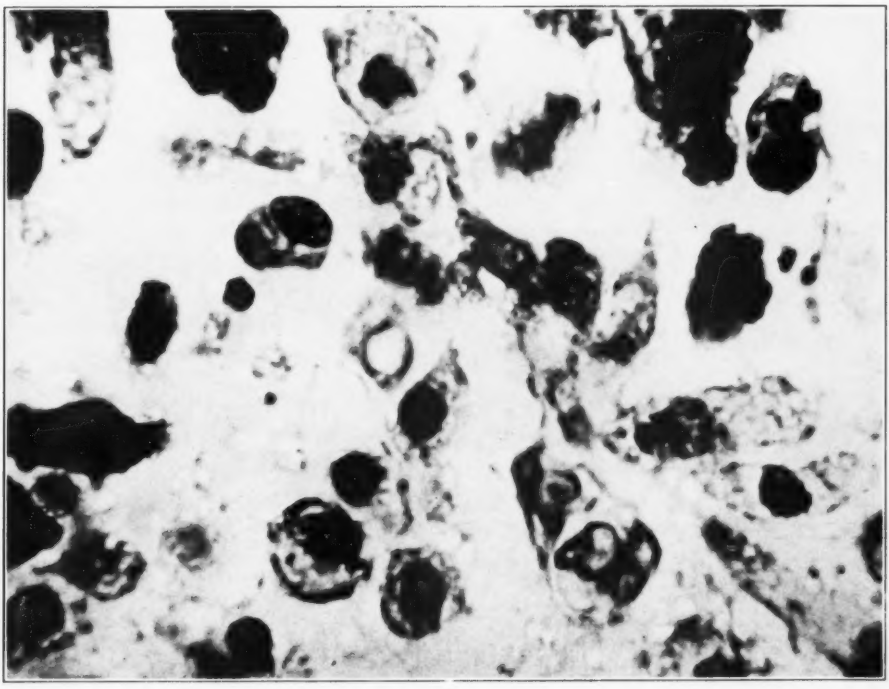

6

Hunter Benign Polymorphocellular Tumors of Knee Region

THE CIRCULATORY PATTERN IN THE ISLANDS OF LANGERHANS *

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The first complete description of the arrangement of the blood vessels of the islands of Langerhans was made by Kühne and Lea [1] in 1882 in the rabbit. By means of injections these investigators found a network of capillaries in the islands which resembled a glomerulus. They also described multiple afferent arterioles, efferent venules and anastomoses with the adjacent interacinar capillaries. They used only single injections and studied the anatomical arrangement by means of sections. Since the work of Kühne and Lea, numerous investigators have studied the circulatory pattern of the islands in various species of animals with contradictory results. Some maintained that the circulation was entirely venous, others that it was entirely arterial, and some that it was only capillary in nature. A complete bibliography of the work done in this field up to 1906 will be found at the end of Dewitt's comprehensive article.[2] Since that time few investigations concerning the anatomy of the circulation have appeared.

In another communication, observations concerning the circulation of the islands of Langerhans in the pancreas of a living white mouse were reported by one of us (B. N. B.).[3] Capillaries, capillary anastomoses, efferent venules, and in some instances afferent arterioles were described. The following anatomical study was undertaken, first to confirm the observations which were made *in vivo*, and second to determine the relationship between the vascular pattern and the preservation of the islands in circulatory disturbances of the pancreas.

Technique

White mice weighing approximately 10 to 20 gm. were used. Under chloroform anesthesia the thoracic and abdominal viscera were exposed through a midline incision extending from the symphysis

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pubis to the manubrium and two lateral incisions extending from the subcostal angle to the axillae. The four flaps formed by these incisions were retracted and held in place by clamps. The body temperature of the animal was kept constant by means of a warm stage that supported a petri dish in which the animal was placed. After cutting away the tip of the heart a glass cannula attached to rubber tubing was inserted into the left ventricle and tied in place. Then a small opening was made in the right auricle to allow the blood to escape during the perfusion.

The injection of the dyes was then started by means of a syringe. Carmine-gelatin, Berlin blue-gelatin and commercial India ink were used. The consistency of the gelatin masses was made to approximate the viscosity of the blood. After trying different combinations it was found that carmine-gelatin followed by India ink was the most satisfactory for double injection purposes. During the perfusion great care was taken to prevent the entrance of air into the system, especially when the change from the red mass to the black mass was made. This was facilitated by an adapter. The interval between the insertion of the cannula and the beginning of the perfusion was made as short as possible to avoid intravascular clotting. The masses flowed easily with little more than gravitational pressure. In order to obtain the correct distribution of the dyes, it was important to observe the vessels of the viscera closely with magnifying spectacles. The ink injection was discontinued when the smallest visible arterial branches of the mesentery and intestines were black and the corresponding veins were still red. It was essential to keep the masses and apparatus warm during the entire procedure. The viscera were kept from drying by irrigating them with warm normal salt solution.

At the end of the perfusion the whole animal was placed in cold 10 per cent formalin. Later the pancreas was dissected out, dehydrated, cleared in methyl salicylate, and observed *in toto*. Selected pieces containing the islands were teased out, or blocks of pancreas were cut with a safety razor blade. These were studied in a small glass dish containing the clearing reagent. Later, the specimens were mounted in balsam or embedded in paraffin and sectioned. The best specimens were the teased preparations. Thick frozen sections were also made.

The Circulation of the Islands of Langerhans

For descriptive purposes it is convenient to divide the islands roughly into three groups according to their sizes (Fig. 1). Group I includes islands more than 0.3 mm. in diameter. Group II, the most numerous, includes those between 0.3 mm. and 0.15 mm. Group III includes the smallest recognizable aggregations of islet cells. In Groups I and II the islands are supplied by one or more arterioles which enter a network of capillaries. The latter anastomose freely with the adjacent interacinar capillaries and empty into one or more venules. In Group III, afferent and efferent vessels are not present.

The Afferent Arterioles: The afferent arterioles are short, varying from 0.06 mm. to 0.18 mm. in length. In diameter they are slightly larger than the capillaries. They arise in most instances directly from the intralobular arteries, and supply the islands in the central zone of the primary lobules. The islands situated near the periphery of the lobules and in the interlobular connective tissue receive short branches from the interlobular arteries. The number of afferent arterioles varies with the size of the islands. In Group I, two or more arterioles are usually present. In Group II, one and less often two afferent vessels are found. In examining a large series of islands, the smallest one supplied by an arteriole measured 0.15 mm. in diameter.

Capillary Network: As the arterioles enter the islands they divide into numerous capillaries which form an interanastomosing plexus. The capillary loops are tortuous, making sinuous curves, spirals and circulets. At frequent intervals, short twigs are given off at various angles forming intercommunications between the loops. This arrangement is characteristic for the islands in Groups I and II. In Group III the capillaries consist of two, three or more tortuous loops which are continuous with the interacinar capillaries.

Anastomoses: Rich anastomoses are found to exist between the capillaries of the islands and those in the interacinar rete. It is easy to distinguish the two types of vessels because the former are shaped in the form of loops, whereas the latter have a pattern which conforms to the outlines of the acini.

Venules: More than one efferent venule is usually present, depending upon the size of the island. The venules resemble the arterioles in that they are short and empty directly into the intra-

lobular veins; sometimes they join the interlobular and larger pancreatic veins. Instead of leaving the island directly, the venules usually follow the surface of the island for a short distance, receiving numerous tributaries from the insular capillaries along their course. In addition, the efferent venules receive tributaries from the adjacent interacinar capillaries.

Discussion

The preceding anatomical studies of the circulatory pattern in the islands of Langerhans confirm the observations which were made *in vivo*.[3] The arrangement consists of a network of capillaries which are supplied with blood through afferent arterioles, empty into efferent venules, and anastomose freely with interacinar capillaries.

An analysis of the vascular system of the islands shows that the arrangement of the vessels favors the circulation of a large volume of blood with maximum efficiency. Since the arterial pressure diminishes directly with the length of the vessel, it is higher at the end of a short arteriole than at the end of a longer one, assuming the diameters to be equal. Therefore the short direct arterioles to the islands afford a higher pressure for the column of blood entering the capillary network than the long arterioles supplying the capillaries of the acinar tissue situated at the periphery of the lobules (see Fig. 2). When the capillary bed of the islands is large, more than one arteriole is present. On the venous side, the multiple short efferent venules of the islands insure a low venous pressure with minimal resistance to the egress of blood. Regardless of the position of the islands in the pancreatic lobules, the dynamics of the circulation remain unaltered. These pressure relationships explain why the islands remain intact in advanced chronic passive congestion, whereas the acini in the peripheral parts of the lobules undergo extensive atrophy.[4] On the one hand, the pressure in the short arterioles to the islands is sufficient to overcome the increased resistance in the venous system. On the other hand, the pressure in the arterioles to the outlying acini is insufficient to overcome the venous resistance which is augmented by stasis in the long efferent venules. As a result, the nutrition of the islet cells remains undisturbed while degeneration of the acini occurs. The few acini which remain intact are found in the immediate vicinity of the islands where the circulation is best.

When thrombosis, embolism, sclerosis or spasm of the afferent arterioles occur, the capillary anastomoses can form a collateral circulation which maintains an adequate blood supply to the islands. This mechanism probably accounts for the few changes which are observed in the islands in the presence of arteriolar lesions.

Examination of the circulatory patterns of the islands of Langerhans in the pancreas of the guinea pig, white rat, monkey and man revealed that they were similar to those of the white mouse.

Summary

1. An anatomical study of the circulatory pattern in the islands of Langerhans was made and the arrangement corresponded with observations made *in vivo*.

2. The importance of the anatomical arrangement of the blood supply in preserving the islands in the presence of circulatory disturbances in the pancreas is discussed.

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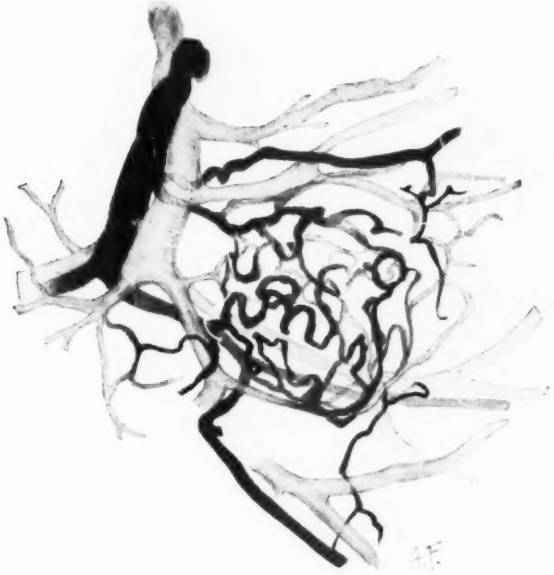
DESCRIPTION OF PLATE

PLATE 6

FIG. 1. Typical arrangement of capillaries in island of Langerhans. Afferent arterioles (black). Efferent venules (gray). Anastomoses with interacinar capillaries. Camera lucida drawing. × 225.

FIG. 2. Schematic drawing of the blood supply of part of a primary lobule of the pancreas showing the circulatory pattern of an island of Langerhans and its relationship to the acinar vessels. (*A*) intralobular artery, (*V*) intralobular vein, (*a*) afferent arteriole, (*v*) efferent venule, (*Is. Lang.*) capillary network in island of Langerhans, (*a'*) small artery supplying acinar tissue, (*v'*) small vein accompanying (*a'*), (*Inter. Cap.*) interacinar capillary network.



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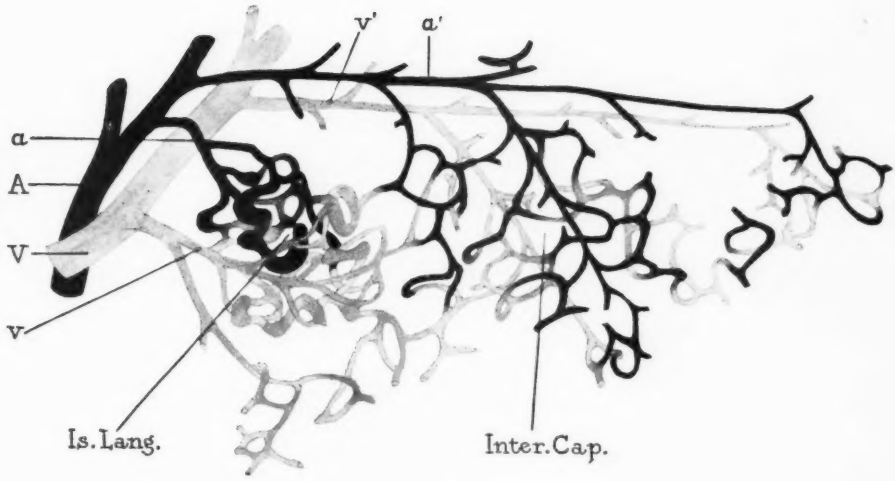

2

INTERACINAR EPITHELIUM OF THE THYROID GLAND *

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The literature concerning the normal and pathological histology of the thyroid gland is extensive and often controversial. In 1863 Virchow [1] wrote that "Die Follikel, welche die letzten Abschnitte bilden, werden gewöhnlich als runde Blasen beschrieben und abgebildet, indess finde ich, dass die scheinbaren Blasen vielfach unter einander zusammenhängen und verästelte, blasige Auswüchse oder Fortsätze besitzen, welche jedoch selten in einer Ebene liegen und daher je nach der Richtung des Schnittes bald isolierte, bald als verbundene, runde, ovale oder längliche Gebilde von sehr verschiedener Grösse erscheinen." In 1880 Wölfler [2] propounded his hypothesis of undifferentiated rests of fetal epithelium to explain the interacinar epithelium so simply accounted for by Virchow as extra-acinar buds. Subsequent studies of the significance of interacinar epithelium have naturally included the configuration of acini and the mechanism of hypertrophy, and many have differed fundamentally in their conclusions.

It is not probable that Wölfler's hypothesis can ever be entirely disproved. Among others, Wegelin [3] and Marine [4] have treated the theory conservatively and have not denied its plausibility. In reference to interfollicular epithelium Marine has recently said, "These cell rests may be considered as the excess of undifferentiated and vegetative thyroid tissue developed during the period of fetal differentiation and normally destined to undergo gradual absorption, but potentially capable of growth and varying degrees of differentiation whenever a sufficient stimulus for increased thyroid activity is applied."

The problem cannot be solved in examinations confined, as in ordinary sections, to one plane. A three-dimensional study is required to establish positively the continuity or discontinuity of interfollicular epithelium with follicles.

* Received for publication September 16, 1930.

This mode of study was first employed by Streiff [5] with the wax plate reconstruction method of Born. Streiff reconstructed a part of a single block of tissue from a normal adult thyroid gland. From an examination of this limited material he corroborated a part of Virchow's observation, as indicated by his statement that "Manche Bläschen weisen sekundäre Ausbuchtungen auf, oder zwei gleich grosse Blasen stehen in offener Verbindung." Without a definite statement as to how he made his tracings, the drawings appended to his article indicate that he traced both internal and external surfaces of acini.

Norris,[6] using the wax plate reconstruction method in a study of fetal thyroids, stated that "Secondary follicles are formed from preexisting follicles apparently by three methods: by solid buds; by hollow buds; and by constriction of the parent follicle." He observed further that although this extra-acinar budding was most active before the fetus had reached 163 mm. in length, it continued throughout fetal life. His reconstructions were also made of both internal and external contours of acini but did not include adult human material.

Wilson [7] studied the thyroid follicle from normal and pathological glands and because he reconstructed internal rather than external contours of follicles, his observations on interacinar epithelium and extra-acinar budding are open to criticism.

Recently Rienhoff [8] has supplemented the wax plate reconstruction method by maceration and microdissection to determine the external configuration of acini. The reconstructions were employed to study the internal form, and the dissected specimens the external forms of follicles. Rienhoff differs from the previous observers in that "There was no evidence of budding, fusion, constriction or division of the follicle when seen and examined in its entirety. . . . It seems evident then that all of these investigations in the past have been concerned chiefly with the individual follicle, and that in them the internal form of the vesicle has been considered the external form or true shape. As a result of this mistake, conclusions as to the continued formation of new follicles in the adult, based on irregularities in the form or shape of the follicles reported in the past are incorrect because the irregularities occur only inside the follicle." He concluded that there were no cell rests either fetal or adult between the follicles and that Wölfler's misconception was based on "tan-

gential sections through the dome of an underlying follicle giving the impression of a group of disconnected epithelial cells."

These investigators (Streiff, Norris, Wilson, Reinhoff) have agreed that there is but little to support Wölfler's hypothesis, but have disagreed fundamentally in regard to the occurrence of external budding of acinar epithelium and the formation of new acini.

Histological Studies of the Thyroid Gland

Blocks of normal thyroid glands, colloid goiters, glands exhibiting active as well as involuting hypertrophy and hyperplasia, nodular (adenomatous) goiters and adenomas, were selected. Many of these blocks were cut in uninterrupted series of sections, some of the series being stained by hematoxylin and eosin and others by the Maresch modification of the Bielschowsky method. It was found necessary to employ the latter method to demonstrate the periacinar reticulum, particularly in hyperplastic glands where the interfollicular stroma had almost disappeared. The serial sections were cut 8 microns thick. To recognize the same structure in succeeding sections of a series, a lobule was chosen as a marker. A lobule could be recognized under low magnification and the change in its contour was sufficiently gradual so that no difficulty was encountered in following it through a series. In blocks of colloid and nodular goiter and in colloid involution of pathological hyperplasia, it was possible to reconstruct from every third or even fourth section, since the changes in acinar contour were more gradual. The drawings were traced from internal and external surfaces of acini from projected images representing a magnification of about 500 diameters.

Wax models were constructed from some of these series. Plates of a thickness corresponding to the magnification were poured and the tracings were transferred to the plates which were then cut. The plates were superimposed to reconstruct the block of thyroid tissue which had been sectioned.

Acinar differentiation was recognized in the thyroid of a 14 cm. fetus, the age of which was estimated to be four months, and which was the youngest fetus included in this study. The thyroid parenchyma was disposed in the form of small, round or irregularly outlined collections of epithelium enclosed by reticulum. Some of these epithelial islands were not solid and contained pale, acidophilic ma-

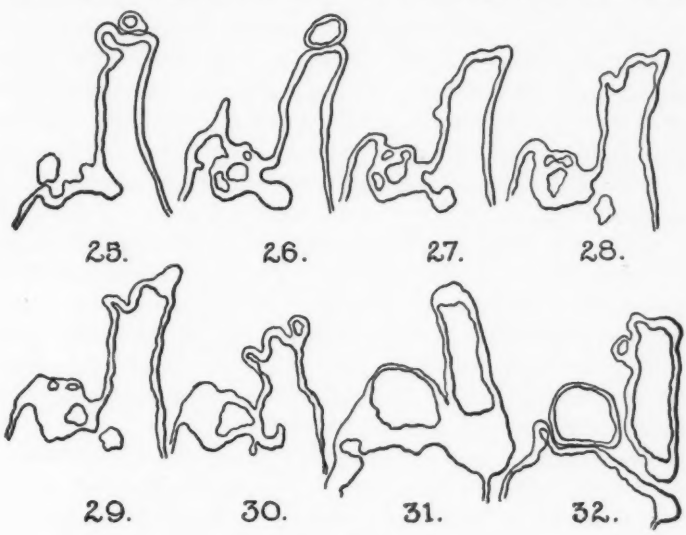
terial. Nuclear polarity did not indicate complete acinar differentiation. Active proliferation was manifested in these cellular islands from many of which buds projected.

In the thyroid of a fetus 20 cm. in length and estimated to be between five and six months of age, acinar differentiation was more advanced. Many of the follicles were lined by cuboidal epithelium exhibiting basal nuclear polarity, were enclosed by periacinar reticulum and contained secretion. Great irregularity in acinar outline was manifest, acini being tortuous, branched structures with solid and hollow extra-acinar buds of epithelium. Such differentiation was not complete, however, and many solid islands of epithelium were encountered. It was apparent that during fetal life new acini were formed from already differentiated acini as well as from undifferentiated epithelium and that the amount of undifferentiated epithelium decreased with the advancing age of the fetus.

A number of normal thyroid glands from children, whose ages ranged from two months to sixteen years, were examined. Some were studied from serial and others from random sections. The islands of undifferentiated epithelium which constituted so prominent a part of the thyroid of the four-month fetus were less conspicuous with advancing age. New acini were formed by intra- and extra-follicular proliferation of epithelium which first formed solid cell masses and later daughter follicles. Solid nests of epithelial cells could be more readily accounted for as the result of extra-acinar hyperplasia of the epithelium lining the acini than as rests of undifferentiated fetal epithelium. These observations were generally in agreement with those of Norris although our studies did not indicate that constriction of follicles occurred. It seemed more likely that the phenomenon described by him as constriction was intra-acinar proliferation of epithelium with formation of secondary follicles within the primary.

In the following consideration of the adult thyroid gland, it may be said that the normal thyroid gland is a labile, everchanging tissue that differs from the pathological gland in degree rather than in kind of change. The definition of a normal gland is necessarily relative and we may say that a gland is normal that conforms in size, shape and structure to the generally accepted average, that generally accepted average to be established from observations in regions not the seat of endemic goiter.

Routine sections of normal (25 to 35 gm.) thyroid glands obtained at autopsy showed that there were scattered foci of actively proliferating glandular epithelium. In a thyroid gland which weighed 30 gm., from a 67 year-old negro who died of syphilitic aortitis, hyperplastic foci were present in what was generally a normal gland. There were areas in which new acini were being formed by intra- and extra-acinar proliferation. Some of these newly formed follicles



TEXT-FIG. 1

Eight tracings of a series from slides which include an area of focal hyperplasia in a normal thyroid. The three follicles seen in section 32 may be traced back to section 30 where their connection with one another is shown. Epithelial proliferation into and out of acini is present. × 125.

had acquired their own periacinar reticulum while others had not and were recognized only by the arrangement of their cells.

A study of serial sections through such an area of focal hyperplasia illustrated clearly the manner in which new follicles were formed and also indicated the origin of what in a random section would have appeared to have been undifferentiated interacinar epithelium. Text-Fig. 1 is a part of a series of tracings from a series of sections of a block including such a focus of hyperplasia. New acini were being formed by solid and hollow buds, some of which projected into the

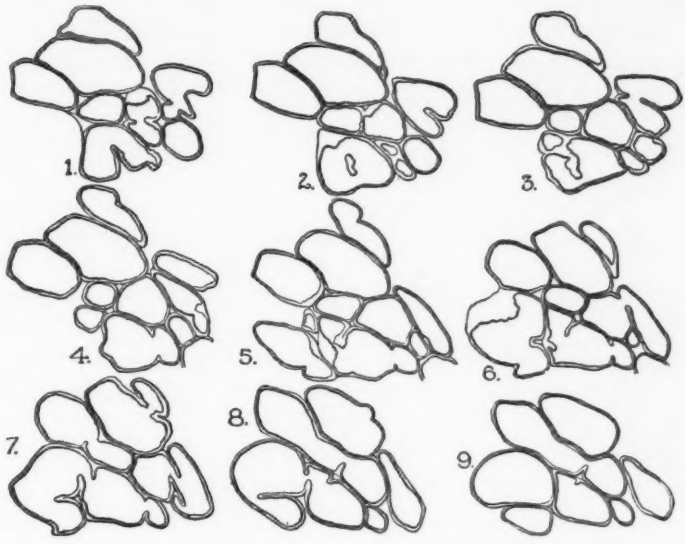
primary acinus while others extended out from it. In section 32 of Text-Fig. 1 there were apparently three distinct follicles which, when traced back to section 30, were seen to be outgrowths of the primary follicle.

The wax model reconstructed from this block of tissue is reproduced in Fig. 1 and the marked irregularity in the external contour of the acinus is seen. Only a segment of the wall of the primary follicle (*a*) is shown, and the follicles marked (*b*) and (*c*) were tubular buds of (*a*). From (*c*) there were a number of small nodular projections of epithelium, some of which were solid and some hollow. The completely detached, solid mass of epithelium (*d*) was supported on a wire strut and may represent undifferentiated interacinar epithelium. It was not possible to prove that such a structure was not a latent rest of fetal epithelium, but it was more easily accounted for as a solid extra-acinar bud which had become detached. Such a cell mass appeared to be a completely undifferentiated and latent structure when stained by hematoxylin and eosin, but with the Bielschowsky method it was seen to have an enclosing reticulum. Often such interacinar epithelium was found to lie within the reticulum enclosing the parent follicle as shown in Fig. 2. It seemed probable that these masses of interacinar epithelium differentiate into follicles which conform to the general structure of the gland.

It was true that most of the solid masses of epithelium encountered in the random section were the result of tangential section through the wall of an acinus and that serial section destroyed the illusion of solidity. Contrary to the observation of Rienhoff, however, there were solid collections of completely detached interacinar epithelial cells.

In agreement with the statement made by Virchow almost ninety years ago, there was intercommunication of follicles to build a labyrinthine system. If it were possible to aspirate the contents of follicle (*a*) in Fig. 1, the colloid would have been withdrawn from follicles (*b*) and (*c*) and others not included by the reconstruction. This does not imply that the entire thyroid is a labyrinthine glandular structure or even an entire lobule. Such intercommunication of acini occurred in areas of hyperplasia. Most of the follicles of the normal thyroid were discrete structures and it was thought that the new acini formed by extra- and intra-acinar proliferation eventually lose their connection with the mother follicle and become discrete.

Intercommunication of acini as a result of coalescence was seen in involuting glands, particularly as a result of iodine therapy. Text-Fig. 2 is a series of tracings of every fourth section from a gland



TEXT-FIG. 2

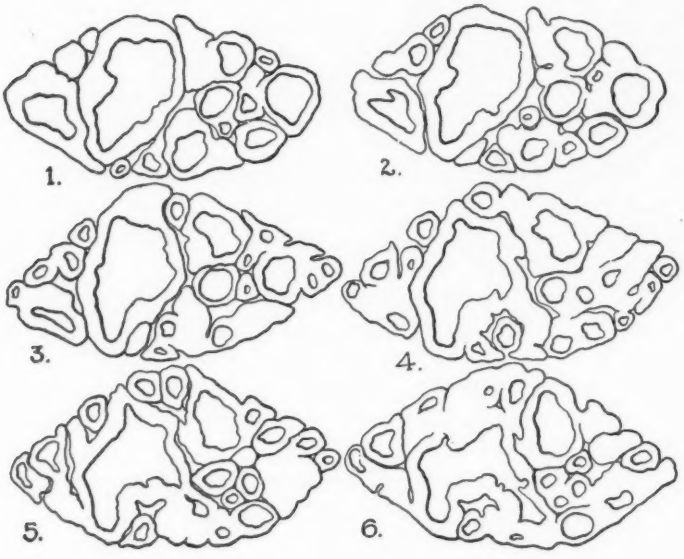
Nine tracings of every fourth section from a series of a block of rapidly involuted thyroid. Confluence of follicles through destruction of acinar walls leaving spurs. × 125.

that had involuted rapidly with rest in bed and iodine administration. Coalescence of follicles was prominent and short spurs marked the site of disrupted acinar walls.

The changes seen in pathological hypertrophy and hyperplasia of the thyroid gland differed from the normal in degree rather than in kind of change. Six tracings of a series through such a gland indicated the complexity of the acinar structure (Text-Fig. 3). Many solid masses of undifferentiated epithelium were encountered, some of which were completely isolated structures while others were readily demonstrated to be epithelial outgrowths from acini. Certainly the structure did not conform to Rienhoff's observation that "It is to be noted that except for minor irregularities in contour, the external surfaces of the follicles of the exophthalmic gland are rela-

tively smooth and as observed in the normal follicles, there are no pseudopodial outpouchings of the epithelial sac, no budding off of new follicles, no constriction or cleavage of the primary into secondary follicles."

That pseudopodial outpouching of the epithelium, budding off of new follicles, and formation of secondary follicles by extra- and intra-acinar proliferation does occur, is shown in Text-Fig. 3 and



TEXT-FIG. 3

Six tracings of a series of slides of a thyroid stained by Bielschowsky-Maresch method exhibiting pathological hypertrophy and hyperplasia. Complexity of acinar structure with intra- and extra-acinar proliferation to form secondary follicles. Pseudopodial buds project from acini. × 125.

Fig. 3, the latter of which is a photograph of a wax model reconstructed from hyperplastic thyroid gland. This is indicated by Fig. 4 which is a section of hyperplastic thyroid gland stained by the Bielschowsky-Maresch method.

Precisely the same sort of growth activity was seen in the nodules of the nodular or adenomatous goiter and in the encapsulated, distinctly neoplastic, so-called fetal adenomas. Because of the serous

degeneration so often seen in the stroma of adenomas, the extra-follicular budding was readily demonstrated by hematoxylin and eosin stains. Such extra-follicular proliferation has been recently described by Boyd.[9]

Summary

1. This study indicates that in normal thyroids of fetuses, children and adults, in pathological glands the seat of hypertrophy and hyperplasia, in nodular goiter, and in adenomas of the thyroid, the mechanics of hyperplasia are essentially similar.

2. New follicles are formed by intra- and extra-follicular proliferation of epithelium and the secondary follicles may lose their parent connection and become isolated units.

3. In the scattered foci of hyperplasia in normal thyroid glands, as well as in glands that are the seat of pathological hypertrophy and hyperplasia, labyrinthine intercommunication of acini exists and is probably limited to the connection of primary with secondary acini.

4. Solid masses of undifferentiated interfollicular epithelium are found in both normal and pathological glands, but such masses are readily accounted for as detached extra-follicular buds.

5. Evidence contrary to certain observations by Rienhoff concerning the mechanics of hyperplasia in the thyroid gland is presented.

I wish to express my appreciation to Dr. H. T. Karsner for his advice and helpful criticism.

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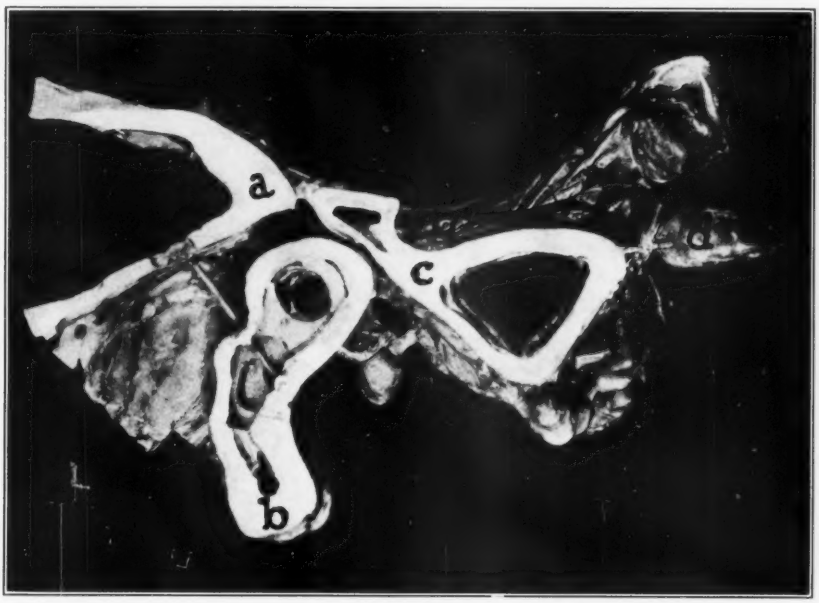
DESCRIPTION OF PLATES

PLATE 7

FIG. 1. Wax model constructed from tracings of serial sections from block of normal thyroid including an area of focal hyperplasia. (*a*) Primary follicle. (*b*) and (*c*) Secondary follicles which communicate with (*a*). (*d*) Solid nest of undifferentiated interacinar epithelium. Model represents an enlargement of 500 diameters. Reproduction is one-half actual size of model.

FIG. 2. Hyperplastic adult thyroid gland. Solid mass of epithelium included within periacinar reticulum. Bielschowsky-Maresch stain. × 300.



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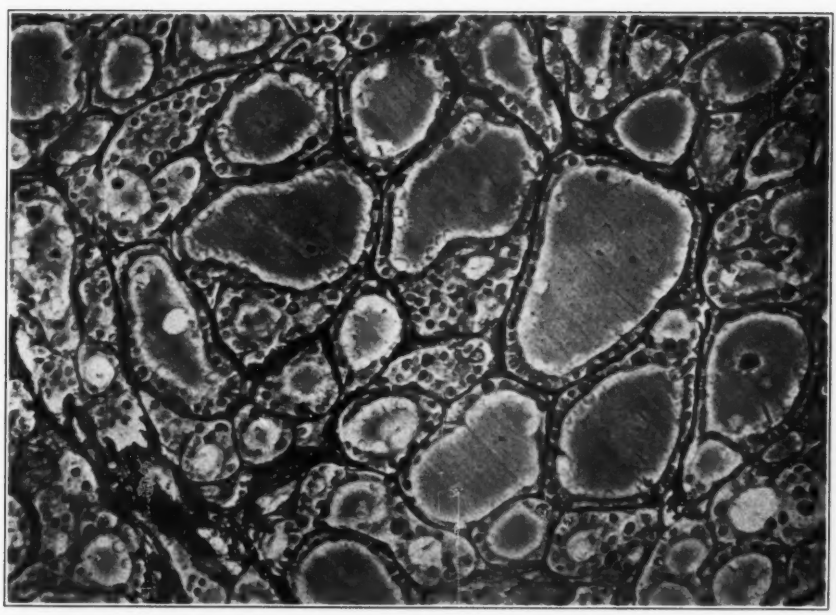

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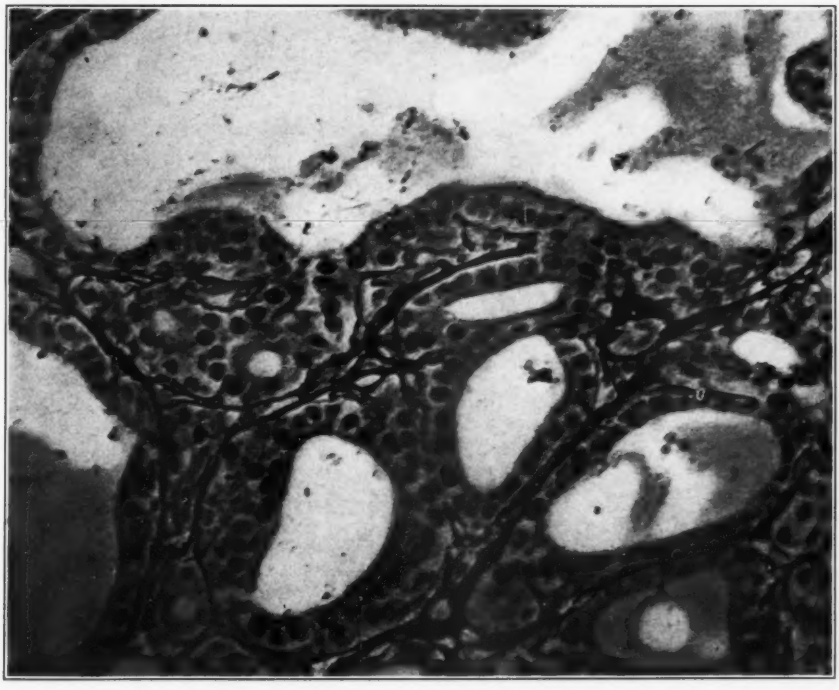
PLATE 8

FIG. 3. Wax model constructed from thyroid exhibiting pathological hypertrophy and hyperplasia. $a - a'$, $b - b'$, $c - c'$, $d - d'$ and $e - e'$ mark interacinar communications. Model represents an enlargement of 500 times. Reproduction is one-half actual size of model.

FIG. 4. Formation of secondary follicles by extrafollicular budding. Some of the daughter follicles have not yet acquired an enclosing reticulum. Small solid bud shows beginning acinar differentiation. Bielschowsky-Maresch stain. × 450.

3



4

Moritz Interacinar Epithelium of Thyroid Gland

PRACTICAL SUGGESTIONS FOR SILVER IMPREGNATION OF CONNECTIVE TISSUE *

L. GUYON

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Impregnation of connective tissue with ammoniacal silver is the most delicate and complex of all histological techniques, but if we take advantage of it, this very complexity renders the method manifold in its applications and fertile in its results. Having acquired a certain skill from long practice, we shall summarize here the lessons of experience.

There are few methods less suited to rigid rules than silver impregnations. To obtain from them all that they are capable of giving, the worker should not rely on strict formulas of time and proportion. The automatic application of no matter what silver technique cannot lead to constant results. To the mishaps thus experienced we may attribute the multiplicity of methods proposed, no one of which is universal or infallible. The picture changes, however, if we bear in mind that the object is not to follow a given silver technique with blind confidence, but to color the sections. This is especially true if we look on each tissue to be studied as a special problem requiring individual treatment with all that this word implies of observation, understanding, interpretation of the facts and adaptation to the actual circumstances.

We shall have attained our object if we can convince the reader that, far from being guided by strict and invariable rules, at each step the worker should survey and know how to interpret the successive results of each manipulation. This is advisable in order to modify, in a direction which it is necessary to discover, the next steps in the technique. No method requires more liberty of procedure, flexibility and initiative on the part of the worker.

FIXATION

In Bielschowsky's original method and in the early techniques inspired by it, the tissue was fixed exclusively in formol. As a matter of fact the methods of impregnation with ammoniacal silver are

* Received for publication October 2, 1930.

sufficiently flexible to succeed, after some modifications, with tissue that has been fixed in many ways. One might think that formol fixation favors the impregnation because there is no intervention of a metallic salt, but such salts as sublimate, chromates and uranium nitrate * do not hinder the impregnation in any way.

Fixation with bichromate-formol-uranium (Tupa's fluid)† has enabled us to impregnate the reticulin of the interscapular brown fat (hibernating gland) of the white rat. The impregnation was particularly successful in the superficial zones where this excellent but slightly penetrating fixative acted correctly. This fluid has given us the best fixation, the most perfect preservation and the purest impregnation of the finest fibrils of the subcutaneous connective tissue.

With Zenker's fluid, Laidlaw obtained his well known brilliant results. We have used Zenker's as well as Helly's fluid and have found them very satisfactory.

Bouin's fluid, even when it has acted for an incredibly long time, permits the impregnation of the reticulin with no preparation other than the removal of the picric acid by thorough washing in running water. This is shown in Fig. 3, elephant's liver that had been preserved in Bouin from 1923 to 1930, an anatomical specimen which we owe to the kindness of Professor Anthony of the Paris Museum of Natural History.

With the piece of elephant's liver, Professor Anthony gave us tissue from another anatomical specimen, a hippopotamus liver that had been preserved in formol since 1917, that is to say, for thirteen years. The disorganization of his service during the war had not permitted as prompt an autopsy as would have been desired. At the time of fixation the liver was already in such advanced putrefaction that bubbles of gas have left their mark in the tissue. Despite such unfavorable conditions, the reticulin impregnates well with no preparation other than treatment of the sections for a week with Gram's solution. The preparations thus obtained were finer than those from the other half of the piece which had been subjected to rejuvenation by ammonia water followed by refixation in Zenker's fluid, as advised by Davidoff.

* Already used by Cajal, but with silver nitrate, not with ammoniacal silver.

† Potassium bichromate 3 per cent..... 80 cc.
Formol..... 20 cc.
Uranium nitrate..... 1 gm.

Passage of the tissue through 4 per cent sodium hydrate, which destroys the protoplasm leaving nothing but the framework, does not in any way hinder the impregnation of the reticulin after fixation in formol or in Helly's fluid, results contrary to those obtained by Foot who was convinced that there is an argyrophil substance dissolved and removed by the soda.

Finally, the reticulin impregnates just as well in tissue that has not been fixed at all. Frozen sections of fresh tissue received in Locke's solution, then washed in distilled water, give very pure and perfect impregnations (Fig. 1).

Laidlaw has shown the importance of regulating the duration of the fixation to avoid the staining of confusing elements such as the neurokeratin of the nerve fibers. Our fixation times have been the usual ones, 6 to 12 hours in bichromate-formol-uranium, Zenker's or Helly's fluids, 1 week in formol, 2 to 3 days in Bouin.

Cutting the Sections

Sections may be made in the usual manner. After formol fixation we make frozen sections, for formol does not fix the tissue hard enough to prevent damage to the finer fibrils by the heat of paraffin embedding. After the other fixatives, embed in paraffin or celloidin. Paraffin would seem to be the easier, but celloidin, just because it avoids the heat of the paraffin bath, is more sure to preserve the finest fibrils. Besides, celloidin permits thicker sections, exhibiting more of the framework, and allows the examination of layers quite distant from the surface of the section, which is always more or less disarranged. This is a great advantage, on condition of course that the preparations are very transparent. If, after fixation, one-half of Ranvier's edematous tumor be embedded in paraffin and the other half in celloidin, comparison of sections from each half will leave no doubt on this point.

The thickness of the sections should be proportionate to the density of the tissue, 10 to 15 microns for nerves, muscle, spleen and brown fat, 20 microns for liver with its larger cells, 30 to 40 microns for white fat, 50 microns for the edematous tumor.

To prevent floating off in the hot silver, paraffin sections are stuck to the slide with gelatin, made insoluble during drying by formol fumes (Masson's method).

Preparatory Treatment of the Sections

Frozen sections of formol-fixed tissue are simply washed in three or four changes of distilled water to remove all trace of the fixative. Sections of Bouin-fixed tissue are washed in running water until the picric acid is removed completely, then passed through distilled water. Sections of tissue fixed in Helly's, Zenker's or bichromate-formol-uranium solutions, and sections that have been preserved for a long time in formol, no matter what the cutting method (frozen, paraffin or celloidin), are treated with iodine in the form of Gram's solution. The sojourn in iodine, never less than overnight, may be prolonged with advantage to three or four days or even one week. The iodine is removed by passage through sodium hyposulphite followed by thorough washing in running water.

When dealing with cells or their prolongations with a particular affinity for silver, such as those of the spinal ganglia or the nerves, or even in tissues where many small cells with their black nuclei tend to mask a delicate reticulum, as in the splenic pulp, it is well to follow the iodine and hypo with potassium permanganate and oxalic acid, as used by Foot and Laidlaw. This treatment, which aims at eliminating the chromate fixed on the protoplasm, diminishes the affinity of the cytoplasm and the nuclei for silver and accentuates the desirable contrast between the reticulin and the background. It should be followed by prolonged washing, observing the usual precautions to avoid the formation of calcium oxalate if the running water at hand is calcareous.

The Silver Bath

All the various methods of preparing ammoniacal silver are about alike. The best is that with which one has the most experience. Knowing its resources and its weaknesses, the worker will know best how to adapt it to each preparation.

For a long time we precipitated the silver with sodium hydrate. To 15 cc. of a 10 per cent solution of silver nitrate, add strong solution of sodium hydrate of any convenient concentration until no more precipitate is formed. Wash the precipitate with distilled water 7 or 8 times, shaking well and letting the precipitate settle to the bottom of the tube each time. The excess sodium hydrate and the newly formed sodium nitrate are thus eliminated. The precipitate,

suspended in 30 cc. of distilled water, is dissolved with ammonia, added very slowly and gradually in the course of an hour. To be sure not to add ammonia in excess, we avoid dissolving the last particles of precipitate, removing them by filtration just before use. Add distilled water up to 60 cc.

At present we prefer a silver solution prepared with lithium carbonate. It has seemed to us that ammoniacal silver carbonate is more stable and keeps for a longer time than the ammoniacal silver hydroxide obtained with sodium hydrate. Dissolve 5 gm. of silver nitrate in as little distilled water as possible and add a saturated solution of lithium carbonate in distilled water until there is no more clouding of the supernatant fluid. Wash the precipitate 7 or 8 times before dissolving it with ammonia. Add distilled water up to 50 cc. Dilute to about one-fourth strength just before use.

The dilute solutions are better than the stronger mixtures. They can be left to act for a longer time, permitting better management of the effect. The silver oxide obtained with sodium hydrate has seemed to us less stable and in consequence more rapid and intense in its action than the silver carbonate prepared by Rio-Hortega's method, using either sodium carbonate or lithium carbonate.

Silver Impregnation

To reveal particularly delicate structures such as the finer fibrils of the subcutaneous connective tissue which are infinitely more delicate than the reticulin of such organs as the liver and spleen, the impregnation is best made at room temperature. The results are incomparable.

Celloidin sections are always better treated cold. With equal intensity of coloration, when stained in hot silver the celloidin is likely to become brittle, not always without risk to the preservation of the tissue. It is much better to let the silver act slowly, more than an hour perhaps, at room temperature in small uncovered dishes. If the dish is covered the impregnation takes place still more slowly. After a certain time a film of metallic silver is seen to form on the surface because the ammonia which held it in solution has evaporated. As soon as the film appears the sections should be passed on to the reducer. In waiting longer there is little gain in intensity of the impregnation, while there is risk of precipitation on the upper surface of the section with more tenacious insolubility of the celloidin.

Frozen sections and paraffin sections stuck on the slide are treated hot, always in a closed vessel and at a temperature no higher than 50° C for fear of serious damage to the tissue. Covering the vessel prevents too rapid evaporation of the ammonia with consequent precipitation of metallic silver and the formation of a film on the surface of the fluid before impregnation is complete.

It is illusory to give precise times for the treatment of the sections with the silver solution either in the oven or when heated over a Bunsen flame with interposed wire gauze. The time varies not only for each fixation and for each organ but also for each specimen of the same organ removed from different animals of the same species, and all the more with different species. We must depend on the color assumed by the sections, which varies with their thickness and the richness of the tissue in cells and consequently in nuclei. The only satisfactory method is to put several sections from the same block in the same silver bath, to begin reduction tests when the sections have become medium brown, and to regulate the procedure by the result of these trials.

Rinsing

Rinsing the sections after the silver bath and before reduction in formol is the most delicate step in the method and the one in which the end results may be best controlled by appropriate modifications. The ideal end result is an almost exclusive impregnation of the reticulin traced sharply on a background as colorless as possible, in which only the nuclei are visible or, rather, outlined. In rinsing the sections the chief object is to remove the excess of silver solution which, reduced by the formol, would give superficial precipitates that obscure the details. At the same time, the rinsing should leave a little ammonia in the depth of the tissue, a procedure that is very important for perfect reduction. In fact, it is indispensable that the sections should be impregnated with a certain quantity of ammonia when they come in contact with the reducing formol.

The rinsing, then, should be just sufficient to remove the excess of silver without removing this necessary ammonia. If prolonged, there is danger of modifying the character of the reduction. The simplest way to attain the desired result is to rinse the sections in weak ammonia water before reducing in formol. The usual mixture is 5 drops of ammonia to 50 cc. of distilled water. Since the ammonia

decolorizes the impregnation somewhat, as shown by bleaching the sections, it is advisable to overstain a little. The bleaching with ammonia is distinctly selective; the nuclei and especially the protoplasm are decolorized considerably before the reticulin is affected. This selective action of ammonia water should be resorted to whenever there is difficulty in obtaining a strongly colored reticulin on a pale background (Figs. 2 and 4).

The strength of the ammonia water and the time of rinsing should be in proportion to the thickness of the section and the richness of the tissue in cells. Thin sections of loosely-woven tissue should be rinsed quickly, about 5 seconds, in very weak ammonia water, only 2 or 3 drops to 50 cc. Sections of open networks, such as liver or spleen that have been treated before fixation with sodium hydrate to destroy the protoplasm, preserving only the framework of reticulin, should be simply dipped in the ammonia water and removed immediately, passing to the reducer without loss of a second. Often it is better not to rinse them at all. In this event, to prevent the precipitation favored by such brief or omitted rinsing, a weaker solution of formol, 1 per cent, should be used. With thick sections of dense tissue such as liver and spleen fixed in the usual way, the ammonia should be increased to 7 drops and the rinsing may be prolonged to 10 or 15 seconds, until the section is distinctly but slightly lighter in color. With comparatively impermeable tissue, such as the clots of artificial collagen obtained according to the directions given by Nageotte, the rinsing must be prolonged and the formol solution should be rather weak to prevent as much as possible the formation of precipitates in the deeper layers of the sections, the layers where the rinsing has not sufficiently removed the excess of silver. The superficial layers, subjected longer and more directly to the action of the ammonia, will usually be rinsed too much and will appear less deeply colored than the deeper layers, where the best pictures should be sought.

Reduction

Reduction is carried on in a more or less concentrated bath of formol. The concentration of the formol should be regulated by the duration of the rinsing and by its intensity, which depends on the strength of the ammonia water. Moderate rinsing of 10 seconds or so in water containing 5 to 7 drops of ammonia to 50 cc. should be

followed by reduction in 15 to 20 per cent formol. Relatively thick preparations and those of less permeable nature, such as the clots of artificial collagen, are reduced in weaker solutions of formol. The shorter the time of rinsing in ammonia water, the weaker should be the formol solution. Five per cent formol is often advisable. Contrariwise, very permeable and delicate preparations, such as sections of the reticulum of the spleen or liver almost freed from the cellular elements by the action of sodium hydrate before fixation, should be reduced in 1 per cent formol after very quick rinsing or no rinsing at all.

If the formol used is very acid, the ammonia in the wash water should be increased. The object of the usual recommendation to dilute the formol with tap water is to neutralize partially this acidity of the formol. We prefer distilled water for this purpose to prevent the precipitating action of the lime salts being added to that of the formol.

Naturally, the thicker and less permeable the sections, the longer will be the time required for reduction. During the entire time of the reduction, the sections or the slides should be agitated in order to renew the fluid in contact with the tissue and to remove the precipitate which forms around the sections.

Toning and Fixing the Sections

We need not describe the well known technique of toning with gold. The aim of this procedure is merely to give prettier preparations without making them any more instructive. It may even have the serious defect of enfeebling or removing the impregnation of the finer fibrils. With very delicate preparations it is better to omit toning with gold and fixing with hypo and to mount the sections directly from the formol bath.

Mounting the Sections

Paraffin sections are mounted in the usual manner, a quick rinse to remove the formol, dehydration in 90 per cent alcohol, then absolute alcohol, xylol, balsam.

Frozen sections are washed and drawn up on a slide prefectly free from grease which is slipped under them in the wash water. The section is spread in a suitable position on the slide, held in place by a

needle applied to a point on its upper border (a point in the preparation which is sacrificed) and the slide is drawn gently from the water, avoiding wrinkles. Dehydrate, observing the precautions necessary with sections unattached to the slide.

Celloidin sections should be washed more carefully for the reducing formol seems to make the celloidin more difficult of solution. The section is drawn up on the slide, care being taken to apply to the slide the surface that was uppermost in the reducing bath. This surface often bears non-adhering precipitates deposited there during reduction and for a few minutes thereafter. If this surface is placed undermost, there is a chance that the precipitate will be rubbed off during the dissolving of the celloidin. If this precaution be neglected the precipitates are liberated during the removal of the celloidin but for the most part they fall back on the section, adhere to the network and can no longer be removed.

The celloidin is dehydrated with absolute alcohol and dissolved by alcohol-ether frequently renewed. If this is ineffective, use acetone. The worker should not be surprised by the rapid evaporation of the acetone, which volatilizes much more quickly than alcohol-ether. Before using acetone, the section should be dehydrated thoroughly for the action of acetone on sections taken from water is disastrous. The acetone, which is rarely perfectly anhydrous, is followed by another absolute alchohol, then by an essential oil (oil of cajeput) and finally by xylol, or directly by carbol-xylol followed by a final rinse in pure xylol before mounting in balsam.

Information Furnished by Preliminary Trials of Reduction

In silver impregnations, one can never be sure of succeeding in the first trial. The first section is reduced empirically and the results obtained serve as a guide in treating more judiciously but without delay other sections of the same batch that have been impregnated along with it.

The nuclei are the first elements stained by ammoniacal silver. After washing in ammonia water and reducing, coloring of the nuclei alone means that the impregnation has not been carried on long enough. The other sections of this batch should remain longer in the silver bath.

Overstaining of the cytoplasm may indicate insufficient washing of the sections before immersion in the silver bath, which, in this event, is clouded too much and too quickly by the formol carried along by the sections; or it may mean insufficient washing in ammonia water before reduction. Among the overstained cells, the fine reticulin is distinguished with difficulty.

If, after reduction, normal reticulin appears as a row of tiny black granules instead of clean-cut brownish black filaments, the ammonia water has been too strong and its action too prolonged. Reduce both the time and the concentration.

A general pale brown reduction indicates too prolonged rinsing after silver. If the sections are particularly thin and permeable, a trial may be made of omitting the washing; then reduce in weak formol, agitating the sections constantly in the reducing fluid.

Occasionally after the silver it is well to wash the sections for some time in distilled water and then dip them for a second in ammonia water before reduction in moderately strong formol, about 10 per cent. The connective tissue bundles will be impregnated black.

In truth, each piece demands special treatment, to be obtained by varying this or that step of the technique, the preparatory treatment of the sections, choice and concentration of the silver bath, temperature, duration and intensity of the impregnation, rinsing, and concentration of the formol reducer. With each specimen, long experience with silver impregnations is the only guide as to which side of the line, by trials, we must seek the lucky modification that will produce the result desired.

DESCRIPTION OF PLATE

PLATE 9

FIG. 1. Calf's liver. No fixation whatever. Frozen section 15 microns thick. × 250.

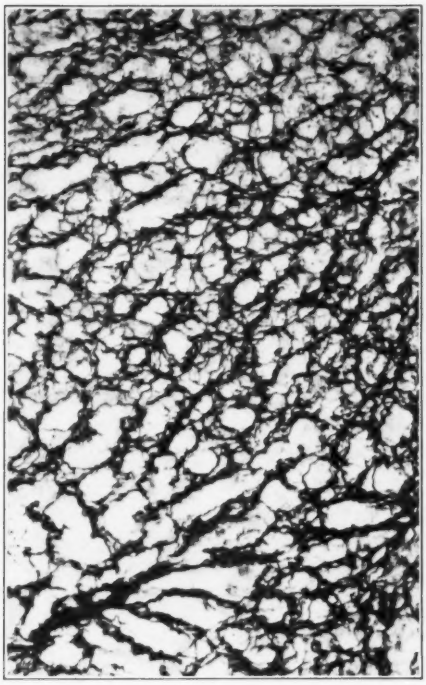
FIG. 2. Horse's spleen. Fixation: Zenker's fluid. Paraffin section 10 microns thick. × 500.

FIG. 3. Elephant's liver. Fixation: Bouin's fluid from the year 1923 to 1930. Paraffin section 10 microns thick. × 250.

FIG. 4. Dog's liver. Fixation: 15 per cent formol. Frozen section 20 microns thick. × 250.

(A) Impregnation in ammoniacal silver carbonate, rinsed in *ammoniacal* distilled water, reduced in 10 per cent formol.

(B) Same impregnation for same time but rinsed in *pure* distilled water, reduced in the same 10 per cent formol.



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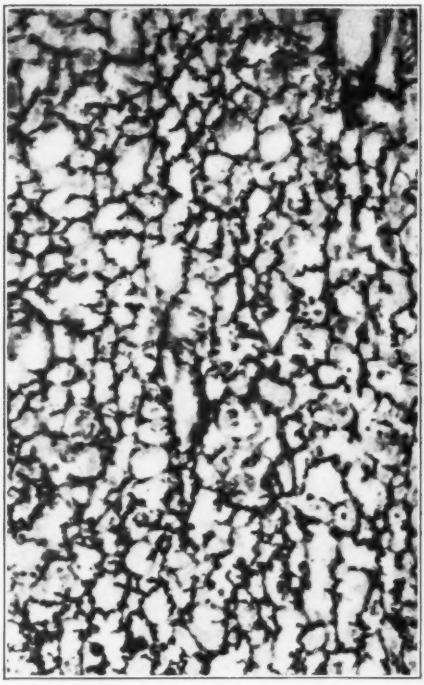

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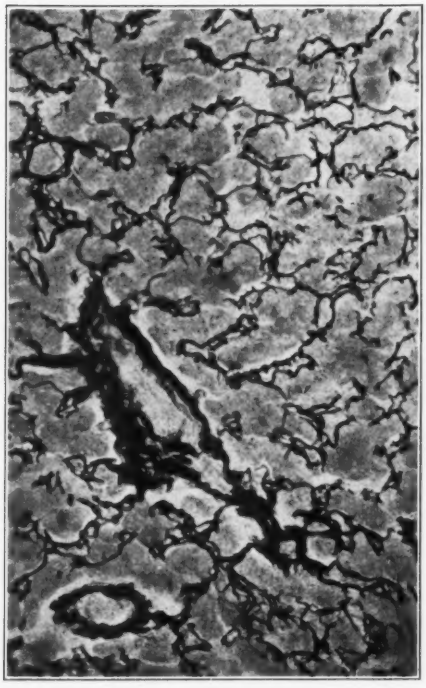

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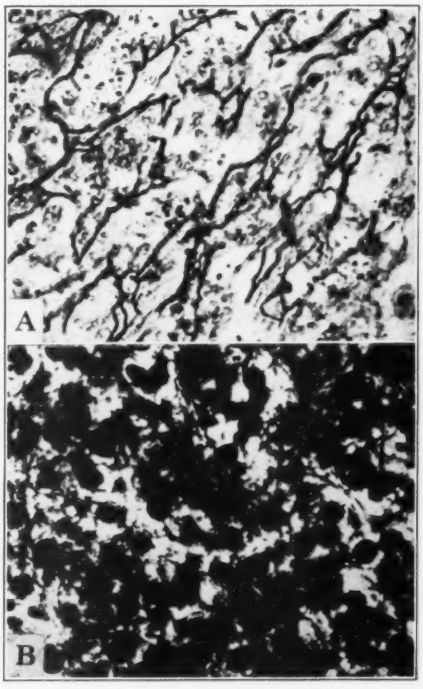

4

Silver Impregnation of Connective Tissue

EXPERIMENTAL GLOMERULONEPHRITIS IN A MONKEY *

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This experiment deals with a monkey which was given repeated intravenous injections of streptococci over a period of four years. It was thought that repeated infections over a long period of time might simulate the conditions under which chronic glomerulonephritis develops in man.

A large male rhesus monkey was selected. Before the experiment was begun the urine was examined repeatedly and found free of albumin, erythrocytes and casts.

The organism used was a stock culture of *Streptococcus viridans* which was originally obtained from the blood of a patient with acute rheumatic fever. The bacteria injected were taken from agar slants incubated twenty-four hours at 37°C. Suspensions of one or more agar slants were made in 10 cc. of normal salt solution. The size of the injection is indicated in the table, + indicating one agar slant, ++ two or three slants, and +++ five slants. The dosage varied somewhat with the amount of growth on the agar.

The urine was collected in a metabolism cage. The animal was not catheterized. The amount of albumin is indicated roughly in the table, + indicating a trace, ++++ when the urine was semisolid on boiling, and ++ and +++ intermediate amounts.

The amount of gross blood is indicated roughly in the table, +++ representing a definitely red color, and + a barely visible redness. Small amounts of blood were frequently checked by microscopic examination.

The injections were discontinued occasionally because of the physical condition of the monkey, since we were anxious to keep the animal alive as long as possible. During the latter part of the experiment particularly, the injections were usually followed by severe reactions during which the animal often seemed near death for several hours. When albuminuria decreased or disappeared, heavier and more frequent injections were given. During the last two years of the experiment only occasional small injections were sufficient to keep up a persistent albuminuria.

* Received for publication October 30, 1930.

Gross blood was present in the urine on 50 out of 119 examinations. It was found much oftener during the latter half of the experiment. The amount of blood was greatest immediately following an injection, after which time it usually decreased gradually.

No edema was present at any time. The blood pressure was not recorded. During the last year of the experiment the animal gradually lost weight and finally became emaciated.

The details of the experiment are given in the following table:

The postmorten examination was made a few hours after death. Definite emaciation was noted. There was no subcutaneous edema, and no fluid was found in the serous cavities. The heart was small, indicating that hypertension had not been present. The lungs were entirely normal. No tuberculosis was present in any part of the body. No disease was found in any organ except the kidneys.

The kidneys were slightly enlarged, weighing together 44 gm. The external surfaces were smooth. On section the cortices were of pale color, not yellowish. Microscopic sections stained with scarlet red showed only occasional fat droplets in the tubules.

Under low magnification a moderate tubular atrophy is found throughout the kidneys. This is indicated by the increased connective tissue between the tubules. The atrophy is diffuse but is more pronounced in some areas than in others. Fig. 1 shows an area with rather conspicuous tubular atrophy.

None of the glomeruli are hyaline, but they all show definite narrowing and occlusion of the glomerular capillaries (Fig. 2). This appearance is present in every glomerulus and therefore cannot be interpreted as a focal lesion. The glomeruli are not enlarged. Very few erythrocytes are seen because of the narrowing and closure of the capillaries. Many of the glomerular lobules have a hyaline appearance.

It is easily seen with the iron-hematoxylin stain (Fig. 2) that there is a widespread obstruction in the glomerular circulation which is sufficient to explain the tubular atrophy. But this stain does not show the details of the glomerular lesion.

Sections stained by McGregor's method (Heidenhain's modification of Mallory's anilin blue) show the nature of the capillary obstruction. In Fig. 3 it is seen that the chief cause of capillary obstruction is a marked increase in the number and size of the capillary

TABLE I

Experimental Data on Monkey Injected with Streptococcus Viridans

Injections		Urine examination			Injections		Urine examination		
Date	Size	Date	Albumin	Gross blood	Date	Size	Date	Albumin	Gross blood
12/21/25	+	12/23/25	−	−	6/3/27	+	6/6/27	+	−
12/28/25	+	..	..	..	6/11/27	++	6/13/27	+++	−
12/31/25	++	1/2/26	+++	−	6/17/27	++	6/18/27	+++	+
1/4/26	++	1/6/26	−	−	..	..	6/26/27	++	−
1/8/26	++	..	..	..	6/27/27	+	6/28/27	++++	−
1/12/26	+	1/13/26	−	−	..	..	6/30/27	++++	+++
1/15/26	++	1/16/26	−	−	..	..	7/1/27	+++	+++
1/18/26	++	1/26/26	−	−	..	..	7/5/27	++	−
1/28/26	++	1/30/26	−	−	..	..	8/30/27	−	−
2/1/26	+++	2/3/26	−	−	9/9/27	++	9/10/27	++++	−
2/5/26	+++	2/5/26	−	−	..	..	9/12/27	+	−
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..	..	11/5/26	+	−	..	..	12/7/27	+++	+++
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..	..	3/3/27	++	−	11/26/28	++	11/28/28	++++	+++
3/3/27	+++	3/5/27	++	−	1/30/29	++	2/1/29	++++	+++
..	..	3/11/27	+	−	7/3/29	+	7/5/29	+++	++
3/26/27	+++	4/24/27	+	−	..	..	7/9/29	Blood urea nitrogen 48.53	
4/25/27	+++	4/27/27	−	−	..	..			
..	..	4/30/27	+++	−	..	..	1/2/30	−	−
..	..	5/3/27	−	−	1/6/30	++	1/9/30	+	−
5/6/27	++	5/9/27	−	−	1/27/30	++	1/30/30	++	−
5/17/27	++	5/19/27	−	−	..	..	5/8/30	++++	++
5/23/27	++	5/25/27	+++	++	Died 7/26/30				

endothelial cells. A few free mononuclear cells are seen which are possibly of hematogenous origin, but nearly all the cells appear to be attached to the basement membrane and are therefore interpreted as endothelial in origin. Only an occasional erythrocyte is seen. The capillary basement membrane is thickened and often appears as a double layer. There is no change in the glomerular epithelium except some evidences of degeneration.

Nearly all the glomerular tufts show a structure similar to that shown in Fig. 3, but those tufts that have a hyaline appearance in the hematoxylin-eosin preparation have a somewhat different structure (Fig. 4). These show a similar increase of endothelial cells, but a very marked increase in the number of layers of the capillary basement membrane. The hyaline appearance of the tuft is clearly due to multiplication of the layers of the basement membrane. Tufts of this structure are no longer permeable to blood.

The renal lesion is therefore characterized by a diffuse proliferation of capillary endothelium and an increase in the thickness and number of layers of the capillary basement membrane. It differs from typical clinical glomerulonephritis in the absence of intracapillary hyaline fibers.[1] There is, however, a notable resemblance in glomerular structure to some human cases of "lipoid nephrosis of mixed type" or "nephritis with nephrotic tendency;"[2] but the similarity goes no further since there is practically no lipoid in the tubular epithelium and there was no edema.

It is highly probable that death was due to uremia. The blood urea nitrogen was 48.5 mgm. per 100 cc. seventeen months before death, but unfortunately it was not determined again. The glomerular obstruction and the tubular atrophy support this interpretation, and no other cause of death was found at postmortem.

In our judgment we have produced a form of chronic diffuse glomerulonephritis which resembles the "parenchymatous" type of the human disease, but does not correspond to any human lesion in all respects. The marked increase of capillary endothelial cells seems to justify the diagnosis of glomerulonephritis.

The lesion was produced by the repeated introduction of streptococci into the blood stream. Whether the bacterial bodies or their soluble toxins are responsible for the injury was not determined. The kidneys were at first resistant to injury, but later became highly

susceptible. This increased susceptibility of the glomeruli is probably merely a response of injured tissue to repeated irritation. It is not necessary to assume that allergic hypersensitiveness existed.

Summary

1. A form of chronic diffuse glomerulonephritis was produced in a monkey by repeated intravenous injections of streptococci over a period of four years.

2. The lesion is characterized histologically by marked increase in capillary endothelium and increase in the thickness and number of layers of the capillary basement membrane.

3. Histologically the glomerular lesion resembles human "lipoid nephrosis of mixed type," except that no fat is present.

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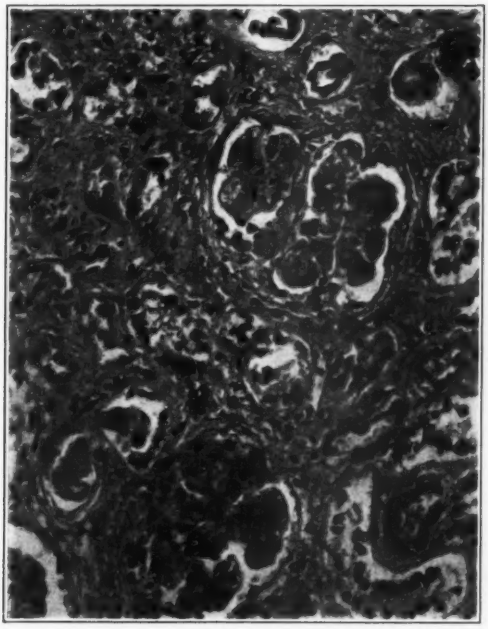
DESCRIPTION OF PLATES

PLATE 10

FIG. 1. Area from the cortex showing moderate tubular atrophy. The glomerular lobules are fused to the capsular layer in some places and their capillaries are partially or completely closed. Iron-hematoxylin stain.

FIG. 2. Two lobules from a glomerulus shown in Fig. 1, under higher magnification. Note occlusion of the capillaries. Iron-hematoxylin stain.



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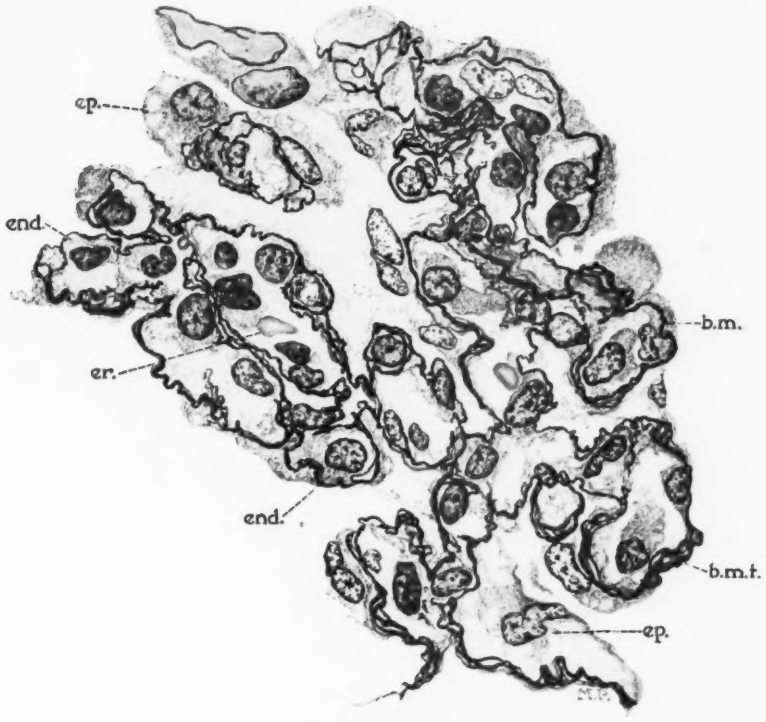
Bell and Clawson Experimental Glomerulonephritis in Monkey

PLATE II

FIG. 3. Area from a glomerulus stained by McGregor's method (Heidenhain's modification of Mallory's anilin blue). Note marked increase of endothelial cells (*end.*). The glomerular epithelial cells (*ep.*) show no changes except evidences of degeneration. The capillary basement membrane (*b.m.*) is thicker than normal and is often represented by two or more layers (*b.m.t.*). (*Er.*) erythrocyte.

FIG. 4. Hyaline lobules from a glomerulus. Stained by McGregor's method. Lettered as in Fig. 3. Note the increase of endothelial cells and the multiplication of layers in the capillary basement membrane.



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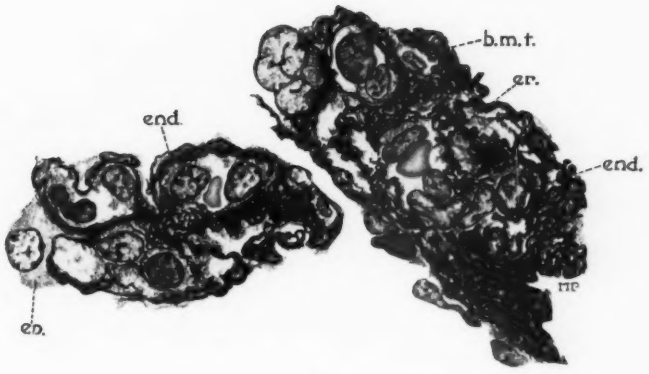

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A CASE OF HYPERNEPHROMA WITH TUMOR THROMBOSIS OF VENA CAVA AND HEART *

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In 1911 Pleasants,[1] in an excellent monograph on the subject of thrombosis of the vena cava, tabulated 29 cases collected from the literature in which the primary source of the neoplastic thrombosis was malignancy of the kidney. Since then other cases have been reported, and in 1925 Simpson [2] after reviewing the literature up to that year found 32 cases. The latter investigator justly points out the futility of attempting to separate the carcinomas from the sarcomas of the kidney in these instances because of the confusion in the terminology by the earlier writers. Intracardiac extension of the tumor thrombi occurred in 13 of these cases, 4 of which were definitely hypernephroma — the cases reported by Sternberg,[3] Wendell,[4] Weber,[5] and Jacobsen and Goodpasture [6] (see table).

Weber's case presented more extensive involvement than any of the others and resembles in many respects the case reported here. In this instance, as in the case described by Weber, the hypernephroma involved the left kidney and adrenal, forming a tumor thrombus which extended along both renal veins, completely occluding the vena cava and involving the iliac veins below, and the heart above.

CASE REPORT

Clinical History: M. S., a white male, 55 years of age, was admitted to the Jewish Hospital of Brooklyn on the medical service of Dr. M. Rabinowitz, April 25, 1928.

Present Illness: Two weeks before admission the patient developed an abdominal swelling and edema of both lower extremities.

Past History: This is irrelevant except for an attack of lobar pneumonia eight weeks prior to admission. Following that attack, there appeared a swelling of the left lower extremity and a similar swelling of the right lower extremity. This condition subsided several weeks before admission.

Family History: Irrelevant.

Physical Examination: The patient appeared quite comfortable, although emaciated. The heart was negative except for the poor quality of its sounds. The lungs were dull to percussion over both bases, especially on the right side.

* Received for publication November 3, 1930.

TABLE I

Cases of Malignant Tumors of the Kidney with Neoplastic Thrombosis of Inferior Vena Cava and Heart

Author	Age of patient	Diagnosis	Involvement of iliac veins	Degree of involvement of inferior vena cava	Degree of involvement of heart
	years				
Barlow and Rose [7]	36	Carcinoma of right kidney	None	From renal veins to heart	Right atrium
Coyne and Troisier [8]	45	Carcinoma of left kidney	None	From level of the fourth lumbar vertebra to heart	Right atrium
Engelken [9]	4	Teratoma of right kidney	None	From renal vein to heart	Right atrium
French [10]	45	Carcinoma of left kidney	None	From renal vein to heart	Right atrium into tricuspid valve
Gairdner and Coats [11]	53	Carcinoma of right kidney	None	Simple thrombus from iliac to renal veins and carcinomatous thrombus from renal vein to heart	Emboli in right ventricle
Jacobsen and Goodpasture [6]	63	Hypernephroma of left kidney	None	From iliac bifurcation to heart	Right atrium and ventricle
Judd and Scholl [12]	29	Adenocarcinoma of right kidney	None	From renal veins to heart	Right atrium
Judd and Scholl [12]	51	Carcinoma of one kidney	None	From renal vein to heart	Right atrium
Kluge [13]	74	Carcinoma of left kidney	None	From renal vein to heart	Right atrium and ventricle
Rosenstein [14]	6	Myosarcoma (cylindroma) of left kidney	None	From renal vein to heart	Right atrium
Sternberg [3]	60	Hypernephroma right kidney	None	From a point about 1.5 cm. above iliac veins to heart	Right atrium
Weber [5]	49	Hypernephroma involving both kidneys	Both veins thrombosed	Complete	Right atrium
Wendell [4]	?	"Epinephroid" carcinoma of right kidney	None	From iliac veins to heart	Right atrium
Wyler [15]	48	Carcinoma of left kidney	Both veins thrombosed	Complete	Right atrium and ventricle
Polayes and Taft	55	Hypernephroma left kidney, extension to the right kidney	Both iliac veins thrombosed	Complete	Right atrium and ventricle

The liver was palpable three finger-breadths below the costal margin. In the lower left quadrant of the abdomen a mass 10 cm. in diameter was felt which was fixed posteriorly, slightly tender, smooth and firm on palpation. The upper and lower poles of this mass could not be palpated. The veins of the right side of the abdomen, as well as chest were quite distended. Those on the left side showed only slight distention. The right lower extremity was slightly edematous.

Laboratory Data:

Blood count: red blood cells 4,950,000 per cmm., white blood cells, 8,200 per cmm., polynuclear leucocytes 74 per cent and lymphocytes 26 per cent. The hemoglobin was 80 per cent (Dare).

Blood chemical analysis: Sugar 107 mg., urea nitrogen 17 mg., creatinine 1.9 mg., and uric acid 4.4. mg. per 100 cc.

Blood Wassermann and Kahn tests: negative.

Urinalysis: Albumin, a trace; sugar, negative; specific gravity, 1.004 to 1.022. Microscopic examination showed numerous red blood cells.

X-ray examination: Flat plate showed opacity not separated from kidney shadow suggestive of either a large or pathological kidney. The other kidney was found to be larger than the average normal kidney. Renal calculi were not visualized.

The chest plate showed a nodule in the left lung suggestive of a metastatic lesion.

Cystoscopic examination: negative.

Pyelogram: negative.

Diagnosis: Hypernephroma of left kidney with thrombosis of the renal vein and inferior vena cava.

The patient was transferred to the surgical service of Dr. J. Linder and a left nephrectomy was performed. The pathological report on the resected kidney was as follows:

Macroscopic Examination: The kidney measures 15 by 8 by 4 cm. The lower pole is the seat of a large nodular mass which invades about one-half of the kidney. The capsule strips with ease, except at those points where it is attached to the nodules of tumor tissue which project through the cortex. There are a few small cortical cysts on the surface. On section the major portion of the tumor is sharply circumscribed. It presents a honeycombed appearance and is made up of a mixture of bright yellow, brown, red and gray tissue.

Numerous smaller nodules of varying size are scattered throughout the rest of the organ (see Fig. 1). The renal vein contains a tumor growth which infiltrates its walls.

Microscopic Examination: The renal structure is distorted and compressed by the tumor tissue which invades it. Large areas are present showing pressure atrophy of renal parenchyma, the latter being characteristically sharply defined from the invading tumor.

The neoplastic structure is typically hypernephroma. It consists of cords of large transparent cells with foamy cytoplasm and small dark nuclei. Large blood vessels in a delicate supporting stroma separate cords of cells. Many of these vessels reach enormous size and some of them have ruptured so that extravasated blood is found at numerous points. Tumor thrombi occlude many of the large vessels. The wall of the renal vein is infiltrated with masses of neoplastic cells and its lumen is occupied by a solid plug of tumor tissue.

Diagnosis: Hypernephroma with infiltration and thrombosis of renal vein.

The patient was discharged after an uneventful postoperative course. About eight months later, however, he was readmitted to the hospital complaining of a recurrence of the abdominal swelling and extreme weakness.

On physical examination he showed the following findings. The lungs were flat to percussion and the breath sounds were absent. The abdomen was distended. A fluid wave and shifting dullness were easily demonstrable. The liver was found to be enlarged, hard and smooth. A small nodular area was palpable in midepigastrium.

Diagnosis: Malignant thrombosis of inferior vena cava with visceral metastasis and ascites.

A paracentesis performed soon after admission yielded 350 cc. of straw-colored fluid which was found to be sterile and to contain many large deeply staining cells with dividing nuclei and many signet ring forms strongly suggestive of malignancy. The patient failed rapidly and several days after admission lapsed into coma and died two days later.

Autopsy Report

The body is that of a well developed and undernourished white male, 55 years of age, with extreme cyanosis of the head and neck, and marked wasting of the panniculus and muscles.

Peritoneal Cavity: Moderately distended by a brownish yellow fluid.

Pleural Cavity: A small quantity of clear yellow fluid on each side, and firm bands between the right lower lobe and posterior wall of chest and diaphragm are present.

Pericardial Cavity: Markedly enlarged.

Heart: Moderate hypertrophy of myocardium in all chambers. The right atrium is markedly dilated and filled with a gray and red friable mass which occludes the right auriculoventricular orifice and extends into the right ventricle. This mass is continuous with a similar one in the inferior vena cava.

Microscopic examination shows edema of the myocardium. The intracardiac mass consists of tumor cells which are attached to the atrial wall and which form an integral part of a thrombus made up of definite layers of platelets and red cells held together by fibrin network.

Inferior Vena Cava: The vessel is dilated to about 5 cm. in diameter by a solid thrombus which extends downward to occlude the iliac veins on both sides, and which fills in the entire lumen of the cava involving the hepatic veins and extending to and continuous with the mass described in the heart (see Fig. 2). The microscopic examination of the thrombus shows a structure exactly like that found in the heart.

Aorta: Normal.

Tracheobronchial Lymph Nodes: Extensive metastatic involvement.

Lungs: Nodular thickenings throughout the parenchyma, made up of soft gray structure traversed by many blood channels. Healed tuberculous lesion in apex of left lung.

The microscopic examination shows nodules to consist of tumor cells, many of which are undergoing mitosis.

Liver: The liver weighs 1220 gm. Surface studded with numerous gray-brown nodules. Hepatic veins filled with gray-red thrombi extending to the main hepatic veins which are occluded by thrombotic masses continuous with that in the inferior vena cava.

Microscopic examination shows diffuse hemorrhages in the parenchyma, especially about the central veins. The main hepatic veins are occluded by platelet thrombi with a few malignant cells embedded in the peripheral portion of the mass, and infiltrating the intimal lining of the vessels.

Spleen: Nodular surface and gray capsule. Vessels occupied by gray-red thrombi.

Microscopic examination shows fibrosis of parenchyma and marked dilatation of veins with areas of diffuse extravasation of blood into the pulp. The thrombi show no malignant cells.

Pancreas: Normal.

Gall Bladder: Normal.

Stomach: Mucosa is hypertrophied and congested. An adenomatous polyp about 4 mm. in diameter is present, situated on the lesser curvature near the pylorus.

Intestines and Mesentery: Normal.

Adrenals: The left adrenal is about 6 cm. in diameter. The cortex is of a normal golden yellow color and the medulla of a gray granular structure with red mottling. The adrenal vein is occluded and lost in the tumor mass which extends from the medulla, involving the major portion of the left renal vein.

The right adrenal is normal.

Microscopic examination of the left adrenal shows complete replacement of the medulla and portions of the cortex by tumor cells. Some of the neoplastic cells seem to arise from the cortex. The right adrenal shows only a moderate amount of hydropic degeneration of the basal cells of the cortex.

Right Kidney: The remaining kidney measures 12 by 6 by 4 cm. The capsule strips easily leaving a fairly smooth surface except for a few cortical arteriosclerotic cysts. The normal markings between cortex and medulla are well preserved. The peripelvic tissue is infiltrated by cauliflower masses which extend into the distal portions of the pyramids at numerous points. These growths are continuous with the masses occluding the renal veins and thus with the main thrombus in the inferior vena cava.

Microscopic examination shows tumor tissue invading the renal parenchyma at the points described above. The renal vein is also occluded by a tumor thrombus.

Right Ureter: The walls are thickened by inflammatory infiltration, but no neoplastic cells can be found.

Urinary Bladder: Hypertrophy of the mucosa.

Genitalia: Normal.

Bones: The ribs, cranium, femora and tibiae are normal.

Lymph Nodes: Neoplastic involvement in the nodes about the bronchi, in the mesentery, at the site of the nephrectomy and in a few of the prevertebral nodes on the right side near the hilum of the right kidney.

Brain and Meninges: Edematous.

Anatomical Diagnoses: Hypernephroma of the left kidney and adrenal with involvement of the right kidney; tumor thrombus in the renal veins, entire inferior vena cava and some of its main tributaries (iliac and hepatic veins) extending into the right atrium and ventricle; lymph node and visceral metastases.

Summary

This is the fifth case of hypernephroma with malignant thrombosis by invasion of the inferior vena cava and heart. It is unique in its extent of involvement. The entire inferior vena cava and all its main tributaries (iliac, renal and hepatic veins) are occluded by a continuous malignant thrombus which extends into the right atrium, through the tricuspid valve into the right ventricle. The cardiac involvement here is even more extensive than in the case described by Weber in which the thrombus did not extend beyond the right atrium. Another interesting feature is the fact that in spite of the massive involvement of the inferior vena cava and the right kidney, the right adrenal gland remained singularly free of metastasis.

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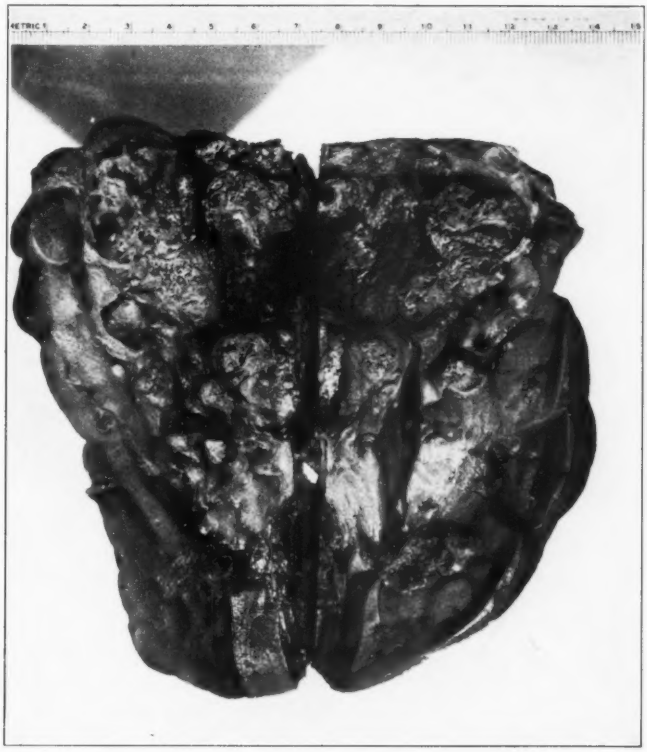
DESCRIPTION OF PLATE

PLATE 12

FIG. 1. Left kidney (removed by nephrectomy) showing extensive involvement by hypernephroma. Note the sharp demarcation between tumor and renal tissue.

FIG. 2. Extension of hypernephroma with involvement of left adrenal and right kidney with neoplastic thrombosis in both renal veins, inferior vena cava, hepatic veins and heart. (Iliac veins have been severed.)



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Polayes and Taft Hypernephroma with Tumor Thrombosis

CHRONIC TYPHOID CHOLECYSTITIS *

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INTRODUCTION

A series of seven cholecystectomies in known typhoid carriers has afforded us interesting material for the study of chronic typhoidal cholecystitis. The duration of the carrier state in these patients varied from three months to thirty years and all but one had been responsible for the infection of other individuals. All of the patients presented symptoms suggestive of either gall-stones or cholecystitis, but in none were the symptoms of sufficient intensity to warrant an operation.

REVIEW OF THE LITERATURE

Although Gilbert and Girode [1] noted the infection of the gall-bladder during the course of typhoid fever as early as 1890, it was not until Chiari's extensive investigation of twenty-two cases in 1894 [2] that the frequency of typhoid infection of the biliary tract in the course of typhoid was recognized. Since then an immense literature has developed upon the subject, much of which is summarized by Garbat [3] and by Gay [4] in their extensive monographs. The great majority of these publications, however, are concerned with the bacteriological or epidemiological features of the condition and in an extensive review of these publications we have been able to find only three in which the histological picture was adequately described.

Chiari, in his original paper, found typhoid bacilli in the gall-bladder usually in pure culture, in nineteen of his twenty-two cases. Thirteen of these showed inflammatory changes, limited in all but one case (and that showed coinfection with staphylococcus aureus) to the mucosa, which showed more or less dense leukocytic infiltration, with hyperemia and edema. In only one case was there necrosis.

In 1913 Bindseil [5] described a single case as follows: "The mucosa was thickened, the folds thick and prominent because of the inflam-

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matory infiltration. The epithelium was largely lacking. The inflammatory cells consisted of lymphocytes, rare polymorphonuclears and large numbers of plasma cells. Sections from the fundus, body and fovea were similar in appearance. Sections from the neck showed a more extensive process with foci of lymphocytes in the muscularis."

Fraenkel in collaboration with Schottmüller [6] in 1925 gave an excellent description of a case. They described a dense infiltration of the mucous membrane of a gall-bladder with lymphocytes and plasma cells. Focal clusters of similar cells were found in the muscularis and along its junction with the serosa. Organisms were demonstrable in the lumina of the crypts but not within the mucosa itself or the other layers of the wall. An excellent photomicrograph accompanying the article shows a typical lymph nodule with hyperplastic germinal center, though this is not mentioned in the text.

Description of Cases

Our own series of cases presented the following findings: In the seven cases of pure typhoidal infection the size of the gall-bladder was within normal limits, although two were moderately dilated. The wall was of normal thickness, thinned, or thickened up to 4 mm. in one instance. The mucosa was relatively thickened in proportion to the other layers; the rugae were prominent and in several cases the mucosal surface was definitely granular in appearance. In no instance was there fibrinous exudate or marked congestion of the serous surface. The content of the organ was in each instance pale, mucoid and clear, or of only moderate turbidity. The color varied from colorless to a light yellowish green staining. Normal bile was not recovered. Stones were found in all but one case. They were usually small, dark in color and crumbled on slight pressure. In two instances they were as large as cherries and facetted. In only one instance was there calcification, and no cholesterol stones were found. Cultures of the bile showed *Bact. Typhosus* in pure culture. In one instance the organism was recovered from the center of a stone.

The microscopic picture varied within considerable limits but certain constant features could be recognized. In all of the cases an inflammatory infiltration was present, always most marked in the

mucous membrane and often sharply limited to it. Usually a few scattered lymphocytes and an occasional lymph nodule were found on the outer border of the muscularis but the process was always very slight in extent compared with that in the mucous membrane. The latter was considerably thickened as the result of a diffuse infiltration of lymphocytes and plasma cells, the former predominating. Just beneath the epithelium the capillaries were dilated and contained numerous polymorphonuclear cells. A few of these cells were found extravascularly in the same region and considerable numbers lay between the epithelial cells, apparently in transit to the lumen of the organ. They were rarely found deeper in the mucosa in the zone of lymphocytic infiltration, although small numbers of eosinophiles were present here in one case.

In addition to the diffuse lymphocytic infiltration, dense focal clusters of lymphocytes in typical lymph nodule formation with hyperplastic germinal centers of immature cells were found in every instance. They were numerous enough to average three to four in each microscopic section and were apparently present in approximately equal numbers in sections from all portions of the wall from neck to fundus.

Microörganisms, though numerous in the bile, could not be demonstrated in any of the layers of the wall. Cholesterosis was not present in any case.

Discussion

Before attempting to interpret the figures in this tabulation the possible sources of error must be considered. On the one hand a false diagnosis of typhoid may have been made, on the other hand three factors would lead to missing positive cases. By all odds the most important of these is inadequacy of the past history either from neglect or because of language difficulties in the many foreign-born patients of a general clinic. Two other definite factors also may play a rôle. "Walking" or abortive cases of typhoid are not very rare and primary typhoidal cholecystitis has been reported by numerous authors. Since the positive diagnosis of typhoid fever is reasonably accurate it seems probable that our figures show too few, rather than too many past histories of typhoid.

Thirty-two, or 8 per cent of our cases gave a history of typhoid fever, and of these, eight or 25 per cent showed the characteristic

histological picture found in our known typhoid carriers. In contrast, of the three hundred and sixty-eight cases with no history of typhoid only eighteen, or 4.9 per cent showed this type of pathology. The five-fold higher percentage seems certainly significant. Assuming that with more accurate histories 50 per cent more positive histories could be obtained, the difference between the two series would

TABLE I

Correlation between Histological Findings and Clinical History in 400 Consecutive Cases of Chronic Cholecystitis

	Number	Per cent
Cases with no history of typhoid	368	92.0
Cases with positive history of typhoid	32	8.0
Cases showing positive "typhoid" histology	26	6.5
Cases showing negative "typhoid" histology	358	89.5
Cases showing doubtful "typhoid" histology	16	4.0
Cases with negative history showing positive histology	18	4.9
Cases with negative history showing negative histology	336	91.3
Cases with negative history showing doubtful histology	14	3.8
Cases with positive history showing positive histology	8	25.0
Cases with positive history showing negative histology	22	68.8
Cases with positive history showing doubtful histology	2	6.2

still be between three- and four-fold. The fact that 68.8 per cent of our cases with positive histories showed no histological evidence of typhoid infection is of course not surprising since only a small percentage of cases of typhoid pass on into the carrier stage.

The eighteen cases with "positive" histology but no history of typhoid are certainly too many to be explained away on the basis of inaccurate histories or unrecognized attacks and we feel that an identical pathological picture can unquestionably be produced by other organisms. The findings of Wilkie [7] and others are interesting in this connection. He found that in the majority of cases of chronic cholecystitis the bile was sterile, while cultures from the outer layers of the bladder wall, or especially the cystic node, were usually positive. *B. typhosus* and a few related organisms grow comparatively readily in bile and it seems possible that the histological picture de-

scribed is typical not of any specific organism but of primary infection of the bile rather than of the gall-bladder wall. This hypothesis would fit the histological picture well, since the lesion in no way resembles the specific histology of the acute stage of typhoid.

Summary

The gross and histological appearance of the gall-bladders of seven typhoid carriers are described. A common histological picture was found. A review of four hundred routine slides of chronic cholecystitis showed a similar lesion in 6.5 per cent of the cases. A review of the clinical histories showed that only 4.9 per cent of the cases with negative histories showed this picture as against 25 per cent of cases with positive histories. It is felt that the lesion described is characteristic of chronic typhoidal cholecystitis but not pathognomonic of it. It is suggested that it is not specific of any organism but represents a reaction to persistent infection of the bile in contrast to the more usual type of chronic cholecystitis with sterile bile and persistent infection of the wall.

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DESCRIPTION OF PLATE

PLATE 13

FIG. 1. A general view of the gall-bladder wall showing dense lymphocytic infiltration of the mucosa, with lymph-nodule formation. × 20.

FIG. 2. A lymph nodule with hyperplastic germinal center. Polymorphonuclears can be seen just beneath the epithelium and between the epithelial cells. × 200.

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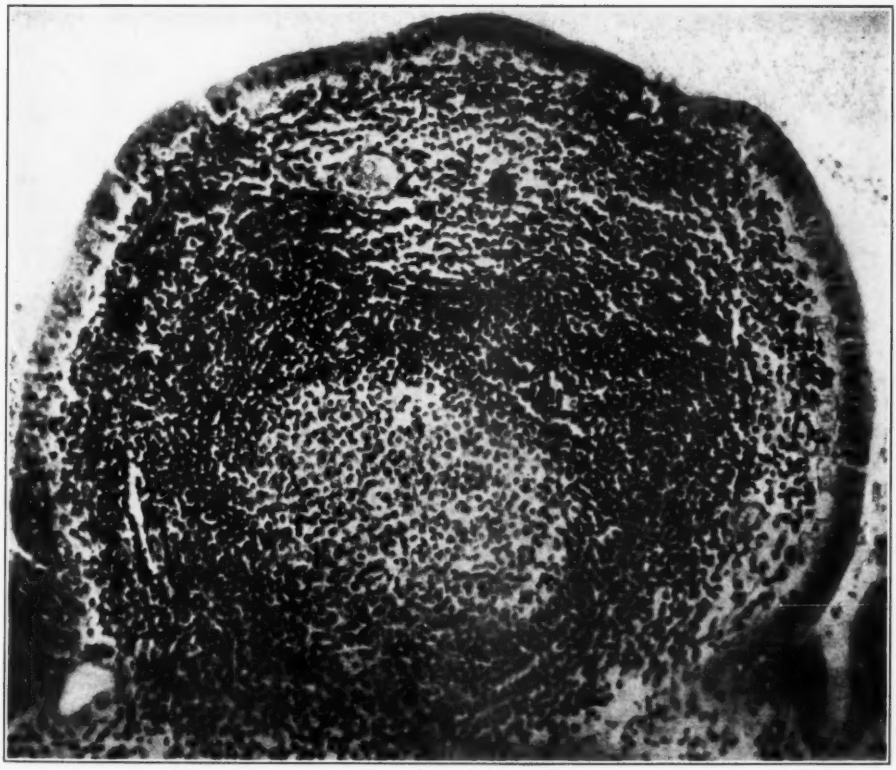

2

Mallory and Lawson Chronic Typhoid Cholecystitis

INFECTIOUS CIRRHOSIS*

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Infectious cirrhosis may be regarded as one of the less common diseases of the liver. It is rare to find it in a pure uncomplicated form. More often it occurs in combination with the obstructive type of cirrhosis. The two types, namely the infectious and the obstructive, occurring either alone or together are often considered as a common entity and grouped together with certain other diseases of the liver under the rather confusing and much misused term of biliary cirrhosis.

It is not the intention of this paper to describe a new and original entity. Infectious cirrhosis is not a new disease: its signs and symptoms as well as its pathological anatomy have long been recognized. One finds, however, in practice, and also in text-books of medicine and pathology considerable uncertainty and misinterpretation in the use of this term. Such confusion may be explained in several ways. First, the disease is not common, consequently the clinician seldom has a fair opportunity to study it; second, the clinical manifestations are rather variable, indeed may be so vague or so slight that an infectious process within the liver is often unsuspected. Similarly, the pathologist who may recognize the disease when seen in its well advanced stages, may overlook it in its earliest form or fail to interpret the varied lesions that one sees in the acute, healing, chronic and healed stages, as nothing more or less than different phases of a single disease. In addition to these facts, one finds the view still prevailing that the terms infectious cirrhosis and obstructive cirrhosis are simply two names for a similar entity; that uncomplicated and prolonged obstruction to the outflow of bile will produce neither recognizable nor constant histological changes; that prolonged biliary obstruction is always complicated with infection within the bile ducts, and that any gross or histological findings that are to be seen within the liver are only the natural result of an inflammatory reaction incited by some form of bacterium.

The purpose of this paper is (1) to define infectious cirrhosis, and to describe it from both the pathological and clinical aspects; (2) to

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point out that infectious cirrhosis and obstructive cirrhosis should not be considered as a single entity but that they differ in respect to etiology, pathological anatomy and in their clinical manifestations, and (3) to show how these two conditions may occur in combination — in which case the pathological and clinical manifestations would depend upon which of the two processes was playing the leading rôle.

LITERATURE

In a paper in 1876 by Charcot and Gombault [1] the term biliary cirrhosis was used to describe lesions of the liver associated with stones in the Ampulla of Vater, cancer of the head of the pancreas and infectious cholangitis. As no attempt at that time was made to differentiate these conditions, the term "biliary cirrhosis" (we adopt Weber's definition [2]) as meaning "any cirrhosis of the liver originating from diseases of the bile ducts or obstruction to the outflow of bile," is quite acceptable.

Heineke [3] in 1897 differentiated two distinct types of biliary cirrhosis; first, one arising from an inflammatory reaction within and around the small bile capillaries and extending outward into the periphery of the lobule, the process being infectious and of an ascending nature. The second type was based on prolonged biliary obstruction.

Mallory [4] in 1911 described infectious cirrhosis as being a type of cirrhosis of the liver resulting from an infection within and around the smaller bile ducts, which corresponds to the infectious type of biliary cirrhosis reported by Heineke.

Rolleston [5] expressed little doubt that the factor which determines fibrosis about the intrahepatic ducts in gall stone obstruction is infection, and regarded the microscopic findings in cases of mechanical biliary obstruction as being of neither clinical nor pathological significance.

MATERIAL FOR STUDY

In a study of the livers from several thousand autopsies performed at the Boston City Hospital, together with a large quantity of material sent in from other hospitals, a total of sixty-five cases was collected showing lesions within the liver which could be grouped as types of "biliary cirrhosis," using again Weber's defi-

nition. Five of this group could be classified as uncomplicated infectious cirrhosis; thirteen were cases of combined infectious and obstructive cirrhoses; thirty-nine were cases of uncomplicated obstructive cirrhosis; while the remaining eight were cases of obstructive cirrhosis that had terminated fatally with an acute suppurative cholangitis with abscesses in the liver. Since this last group showed no cirrhotic changes of infectious origin, they were not included in this series.

Etiology of Infectious Cirrhosis

As the name implies, this type is due to an infection within the terminal bile ducts which invades the adjoining lobules. Cultures of the liver were taken in only two of the five cases and although the colon bacillus was isolated in pure culture from each of these, such evidence is quite insufficient to ascribe to it any definite etiological rôle. However, in one of these two cases microscopic examination showed rather small slender bacilli in the lesions, whereas in the other no organisms could be demonstrated although the lesions had not passed the acute inflammatory stage. Of the three remaining cases, two showed only old healed lesions; therefore, it is not surprising that organisms could not be found. In the last of the five cases, slender bacilli could be demonstrated within the chronic inflammatory lesions. Unfortunately, this case had not been cultured.

A temporary obstruction to the outflow of bile may possibly have been an important contributing factor in the etiology of these five cases, because in each there was a history of a transient, though slight degree of jaundice. However, at autopsy no obstruction was found and a histological examination of the liver showed no lesion suggesting biliary obstruction.

The manner in which organisms reach the terminal bile ducts is probably by an ascending course from the duodenum through the large and small bile ducts. However, the possibility of infection reaching the liver by way of the circulation and lymphatics cannot be entirely disregarded. This phase of the problem has recently been fully reviewed by Brulé [6] who discusses in detail all the possible paths by which infection may reach the liver.

Histological Features of Infectious Cirrhosis

The picture at first is merely that of acute inflammation within the portal areas, resulting from the presence of pathogenic organisms within the terminal bile ducts; from here the inflammatory reaction spreads among the surrounding liver cells (Fig. 1) so that the lesion is confined not alone to the portal areas but also to the peripheral zones of the lobules.

The portal areas which appear to suffer the brunt of the infection are the rather small or medium sized ones; however, even the smallest and largest may at times become involved. Furthermore, the inflammatory reaction may not be equally advanced in all portal areas within the liver so that some may show acute inflammation, others a rather chronic reaction, whereas in others the process may be almost or entirely healed. In order to interpret the histological findings in a liver in which the process has entirely healed, it is necessary to have traced and to know thoroughly the development of the lesion from its early beginning to its end.

The small bile duct is the center of an acute inflammatory reaction; the lumen is distended with polymorphonuclear or endothelial leucocytes (Fig. 4), depending on the number and virulence of the infecting organism. The epithelial cells are stretched and show regressive changes, and the surrounding stroma is infiltrated with an acute inflammatory exudate. As the organisms invade the tissue around the ducts (Figs. 2 and 3) and the process extends, the liver cells in the outer portions of the lobules show retrograde changes; even at this early stage one may find proliferation of fibroblasts. Later, there is a zone of degenerating and necrotic liver cells infiltrated with polymorphonuclear and endothelial leucocytes surrounding the portal area. The endothelial cells lining the sinusoids, and the supporting connective tissue are also damaged so that the normal architecture of the periphery of the lobules is entirely lost.

Very early, the bile duct epithelium begins to proliferate, mitoses are numerous, and any small periportal ducts that have not been destroyed, elongate and give rise to many new ducts which extend out into the surrounding zone of necrosis to link up with the free ends of bile capillaries.

Occasionally one finds the walls of vessels also involved in the in-

flammatory reaction and fibrin thrombi adherent to the damaged endothelium partially obstructing the lumina. The lymphatics in the capsule and large portal areas are filled with endothelial and polymorphonuclear leucocytes, coagulated albumin and fibrin.

In the healing stage, when the process is less active and the bacteria have died out, the exudate consists chiefly of endothelial leucocytes, many of which are phagocytic and contain necrotic cellular débris. Long before this period, as already mentioned, fibroblasts have begun to proliferate, largely as the result of direct injury to the connective tissue by the bacteria and their toxins, but also in part to form stroma for the greatly increased number of small bile ducts.

In the smaller portal areas the lesion spreads very uniformly as an expanding circle from the bile duct toward the surrounding hepatic veins, but in the larger portal areas, where there may be several ducts and where the ducts are so eccentric, the infection may not reach each of the adjacent lobules but may extend out in a semicircular fan-shaped manner destroying the periphery of only one or more lobules along one side. On the whole, however, the true lobular arrangement is more or less perfectly preserved and the portal areas, as a result of the extensive exudate, of the necrosis of the liver cells and of the encroachment and proliferative activity on the part of the terminal bile ducts and connective tissue, show up as broad bands running rather regularly among the lobules of liver cells.

In contrast to the lesion in the acute or healing stages, the healed lesion is much less conspicuous. The necrotic cells have been removed; a few scattered lymphocytes, singly or in foci, are all that remain of the inflammatory exudate; the stroma is shrunken and the small bile ducts are compressed. The portal areas now stand out simply as bands of dense fibrous tissue containing many small bile ducts. The portal vessels are frequently sclerosed, and here and there, lying near the portal areas, are small isolated clumps of liver cells.

Anatomical Features of Infectious Cirrhosis

In the early stage of infectious cirrhosis, the liver is normal in size and contour, the capsule is smooth, the fresh surface may be bile-stained and the portal areas may be slightly accentuated, but there

is so little grossly to suggest the histological changes that the lesion at this stage may be readily overlooked.

As the process becomes more chronic and more extensive the liver enlarges from a third to double its normal size. The capsule is smooth and tense. The consistence is increased and the liver cuts with more resistance. The fresh surface may be a little bile-stained or deeply congested, but the broad portal areas even at this stage are not very clearly defined.

The healed stage is characterized by a contraction of the newly formed stroma so that once again the liver may return to about normal size. The surface is rather uniformly and finely nodular with the nodules sometimes measuring nearly one centimeter in diameter. The consistence is firm and the liver cuts with greatly increased resistance. The cut surface presents a striking picture (Fig. 5). There is a marked increase in the amount of connective tissue about the portal areas so that they appear as grayish white, fibrous bands which may be traced from the transverse sinus to the capsule.

Clinical Aspects of Infectious Cirrhosis

Since these are so dependent on the nature of the lesion within the liver, they will of course vary considerably. The onset may be gradual and unaccompanied by any diagnostic sign or symptom; however, as is so commonly the case, jaundice, perhaps very slight and only transient, is one of the first things to be noticed. Lassitude, drowsiness and gastro-intestinal disturbances, accompanied later by loss of strength and weight are clinical findings common to each case. The temperature may vary slightly from day to day but seldom rises above 100 F. Similarly, the blood count may show no appreciable change, except a slight leucocytosis which sometimes reaches twelve or fifteen thousand cells per cubic millimeter.

In the early stage, as already pointed out, the liver is normal in size; in the chronic and healing stages it is enlarged, while later, if recovery ensues, it may shrink again to normal size and is then uniformly nodular. The spleen may be somewhat enlarged from the onset. As the process in the liver regresses, with a gradual contraction of the newly formed connective tissue, the portal circulation becomes appreciably obstructed so that in the healed stages the spleen may

be considerably enlarged, due to chronic passive congestion. Ascites and esophageal varices were found in one case. In the healed stage there is neither jaundice nor histological evidence of bile stasis.

Summary of the Pathological Findings in Infectious Cirrhosis

The outstanding features of infectious cirrhosis can be stated briefly; a sequence of changes may be followed beginning with an infection within the terminal bile ducts, which soon spreads and destroys the surrounding liver cells. This not infrequently causes damage to the walls of the vessels as well. Bile ducts and connective tissue rapidly regenerate and, with the disappearance of the inflammatory exudate and the necrotic cellular débris, the late characteristics of this type of cirrhosis soon become well established.

Comparison Between Infectious and Obstructive Cirrhoses

First as regards etiology; one results from an infection within the terminal bile ducts, the other from prolonged biliary obstruction. The infectious type of cirrhosis lacks a uniform general distribution, whereas in the obstructive type of cirrhosis [7] the histological changes characterized by an elongation and apparent increase in bile ducts are found in every portal area within the liver. Furthermore, whereas the outstanding features in the early stage of infectious cirrhosis are infection, inflammation and necrosis, in the obstructive type it is the extreme degree of bile stasis which first attracts attention.

Grossly, the liver in uncomplicated cases of obstructive cirrhosis is normal in size, and the surface is smooth except when the obstruction has been of very long standing, and then it assumes a very finely granular Morocco leather appearance.

Lastly, while the classical signs of a severe degree of obstruction to the portal circulation, namely esophageal varices, a large spleen and ascites, are found in long-standing and healed cases of infectious cirrhosis, they are lacking in cases of the obstructive type.

INFECTIOUS CIRRHOSIS COMBINED WITH OBSTRUCTIVE CIRRHOSIS

This combined form of cirrhosis, though much less common than the simple obstructive type, is encountered more frequently than the infectious type alone, and is simply the result of an infection within the terminal bile ducts of a liver that has already begun to show the characteristic changes resulting from prolonged biliary obstruction. Hence, the lesion is merely the picture of one type of cirrhosis superimposed on another, and because the inflammatory process resulting from the infection spreads quickly it soon becomes the outstanding feature both grossly and microscopically (Figs. 6, 7 and 8).

This combined form of cirrhosis may occur at any age; two examples were in infants several months old, one in an adolescent, and the remainder in adults. In our small series males and females were about equally affected. As in the simple uncomplicated type of infectious cirrhosis, the infection probably reached the liver by way of the biliary system; however, since two of our cases were in infants that at the time of the autopsy were considered to show complete atresia of the common bile duct, the possibility of the infection coming from the vascular or the lymphatic system should also be considered. The infecting organism in three of the cases that were cultured was the colon bacillus; unfortunately, no bacteriological studies were carried out in the others, but in eight, bacilli were demonstrable histologically within the lesions.

At this point it is quite unnecessary to repeat the description of the histological lesions of both the obstructive and infectious types of cirrhosis and consequently only the most characteristic findings will be mentioned. The lesions of infectious cirrhosis may be in the acute or chronic stage, but since this combined type of cirrhosis almost invariably ends fatally, a liver showing the combined healed infectious and obstructive lesions is extremely rare and was encountered in only one of our cases, and in this the obstructing agent had disappeared.

One of the remarkable features of this combined type of cirrhosis is the large number of infarcts seen in the periphery of the lobules which resemble in every way those that are occasionally found in the uncomplicated type of obstructive cirrhosis. Sooner or later many of these become infected and are then distended with an inflamma-

tory exudate which compresses the surrounding liver cells. The origin of these areas of necrosis,[8] which occur rarely in simple obstructive cirrhosis and more commonly in the combined form of obstructive and infectious cirrhosis, can be explained on several grounds. First, there is a mechanical interference with the blood supply in these areas as a result of wide distention of the bile ducts compressing the vessels in the portal area; (2) in the process of inflammation there is a slowing of the circulation, and (3) as a result of the injurious agent the vascular endothelium is injured and thrombi are frequently formed. With this explanation these areas of necrosis may be interpreted simply as infarcts which have undergone unusually rapid liquefaction as a result of imbibition with bile.

Another finding in this combined form of cirrhosis is the relatively inconspicuous rôle bile stasis plays in the picture, so that instead of seeing the bile capillaries distended with bile they are collapsed and almost empty. This may probably be explained either by the escape of bile from the free ends of the bile capillaries into the periportal zone of inflammation and necrosis, or on the basis that the liver cells, in the presence of infection, cease to carry out their secretory function, and consequently bile is not being formed.

Comment

Uncomplicated infectious cirrhosis is one of the rarer diseases of the liver. It is caused probably in most instances by the colon bacillus, and is characterized by a variable degree of connective tissue and bile duct proliferation about the portal areas.

Obstructive cirrhosis resulting from prolonged biliary obstruction is a much more common condition although less conspicuous and hence frequently overlooked.

Most cases of infectious cirrhosis are found in combination with obstructive cirrhosis, but even this combined type is relatively rare and is almost invariably fatal.

Summary

1. The gross and histological lesions of infectious cirrhosis, when occurring alone or in combination with obstructive cirrhosis, are described.

2. Infectious cirrhosis is contrasted with obstructive cirrhosis from the pathological and clinical aspects.

3. The literature bearing on infectious cirrhosis is briefly reviewed.

I am indebted to Dr. F. B. Mallory for his help and criticism and for the illustrations.

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DESCRIPTION OF PLATES

PLATE 14

FIG. 1. Periphery of a lobule, showing invasion by colon bacilli, destruction of liver cells and an inflammatory exudate of polymorphonuclear and endothelial leucocytes. The periportal connective tissue is increased in amount. From a man aged 85 years. × 250.

FIG. 2. Invasion of liver parenchyma by colon bacilli: acute inflammatory exudate consisting of polymorphonuclear leucocytes. From the same case as Fig. 1. × 1000.

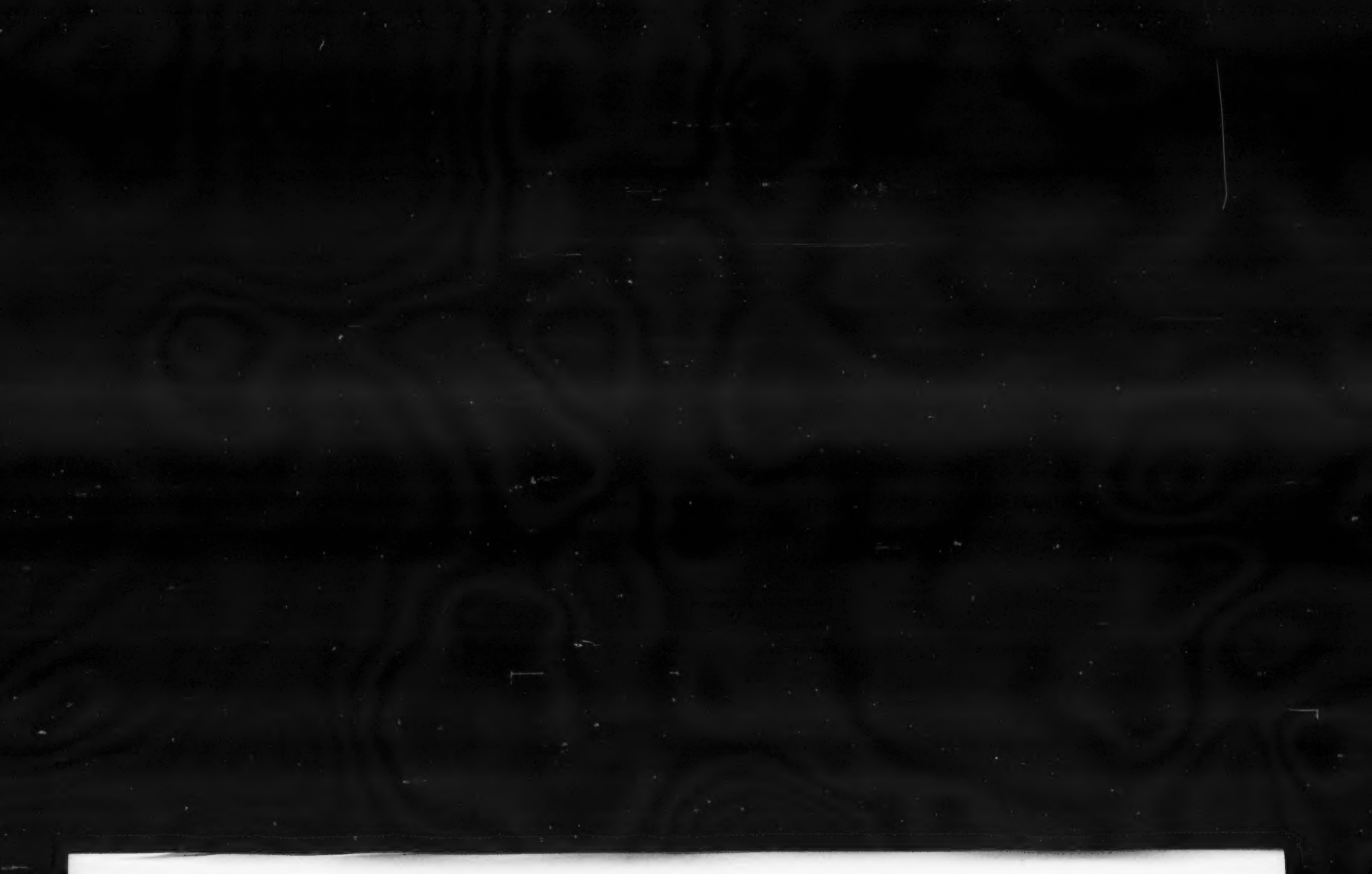
1

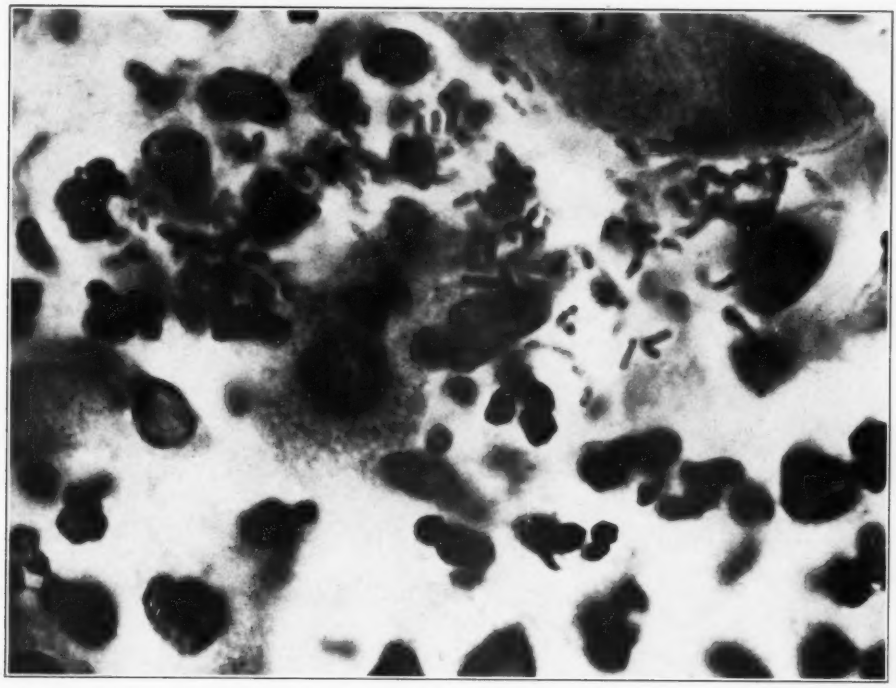
2

Plate 15

Fig. 3. Invasion of liver parenchyma by colon bacilli: acute inflammatory exudate consisting of polymorphonuclear and endothelial leucocytes. From the same case as Figs. 1 and 2. × 2000.

Fig. 4. Bile duct dilated: contains an irregular cylindrical mass of inspissated bile and an exudate of endothelial leucocytes. The surrounding stroma is infiltrated by endothelial and polymorphonuclear leucocytes and a few lymphocytes. From a girl 13 years of age whose liver was twice normal size. × 1000.





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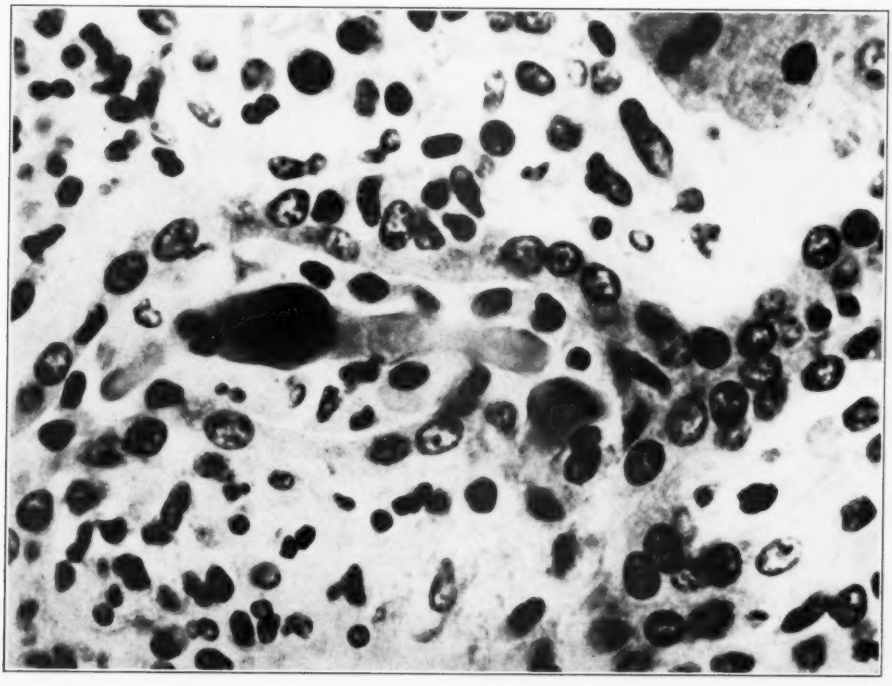
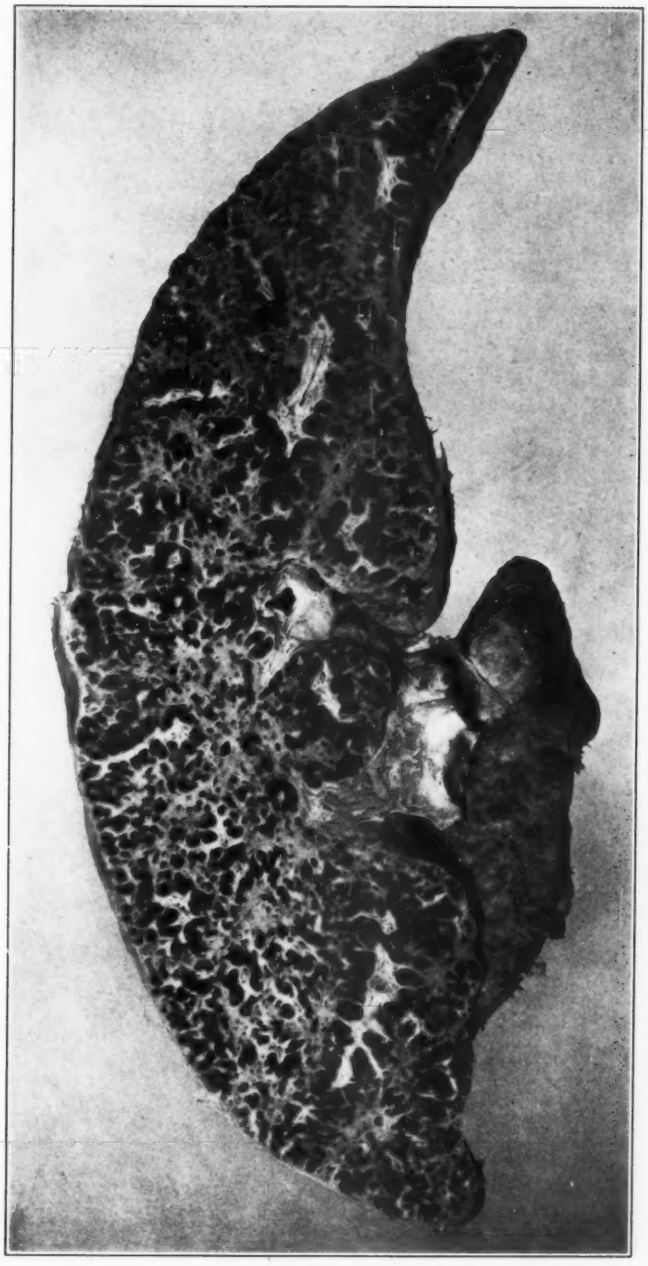


4

MacMahon Infectious Cirrhosis

PLATE 16

FIG. 5. Healed infectious cirrhosis. The lesion is more or less irregularly distributed. From a woman 25 years of age. Weight of liver 1090 gm. Ascites but no jaundice.



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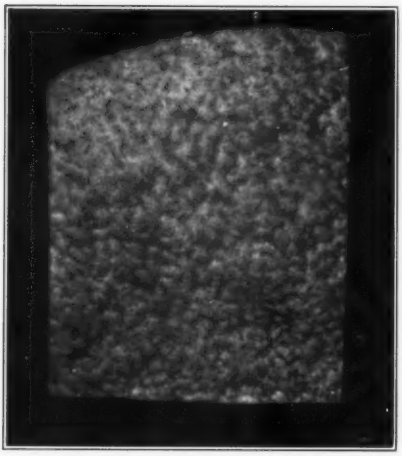
MacMahon Infectious Cirrhosis

Plate 17

Figs. 6, 7 and 8. Healed obstructive and infectious cirrhosis from a man 40 years of age. Weight of liver 2495 gm. Jaundice, esophageal varices. The lesion is very evenly distributed throughout the liver. The surface is granular. The photomicrograph shows that the lesion is situated around the portal vessels. The even distribution of the lesion, in contrast to that seen in Fig. 5, is probably due to the bile stasis which enabled the infecting agent to spread uniformly throughout all the dilated bile ducts. × 50.



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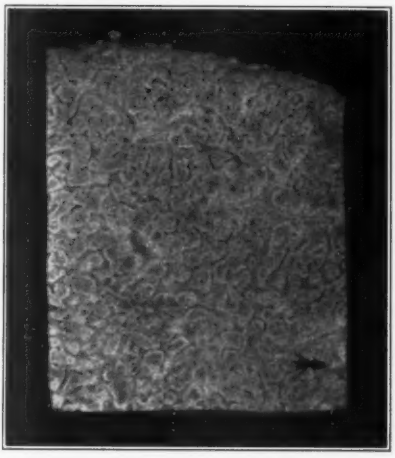

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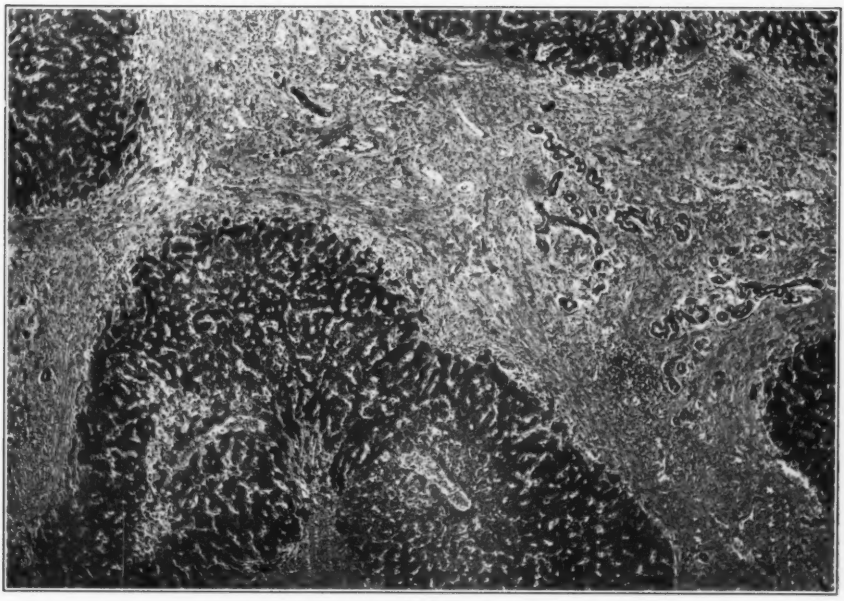

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